Organization TC1600 Bldg./Room REMSEN

U. S. DEPARTMENT OF COMMERCE
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450
IF UNDELIVERABLE RETURN IN TEN DAYS

OFFICIAL BUSINESS

UNITED STATES POSTAGE U.S. OFFICIAL MAIL
PENALTY FOR PRIVATE USE $300
PITNEY BOWES
02 1A $ 01.59⁰
0004204479 APR 25 2006
MAILED FROM ZIP CODE 22314

AN EQUAL OPPORTUNITY EMPLOYER

RECEIVED
APR 27 2006
USPTO MAIL CENTER

UNITED STATES PATENT AND TRADEMARK OFFICE

IFW

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/005,211	12/04/2001	Keith D. Allen	R-325	5578

26619 7590 04/25/2006

JOHN E. BURKE
GREENBERG TRAURIG LLP
1200 17TH STREET, SUITE 2400
DENVER, CO 80202

EXAMINER	
QIAN, CELINE X	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 04/25/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/005,211	ALLEN, KEITH D.04	
	Examiner	Art Unit	
	Celine X Qian	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 March 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *25-30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *25-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *04 December 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 25-30 are pending in the application.

This Office Action is in response to the amendment filed on 3/8/04.

Response to Amendment

The finality of the office action mailed on 12/2/03 is withdrawn.

Claims 25-30 are rejected under 35 U.S.C. 112 1st paragraph for reasons discussed below.

New Grounds of Rejection

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 25-30 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue." These factors include, but are not limited to: (a) the nature of the invention; (b) the breadth of the claims; (c) the state of the prior art; (d) the amount of direction provided by the inventor; (e) the existence of working examples; (f) the relative skill of those in the art; (g) whether the quantity of experimentation needed to make or use the invention based on the content of the disclosure is "undue"; and (h) the level of predictability in the art (MPEP 2164.01 (a)).

Nature of the Invention:

Claims 25-30 are drawn a transgenic mouse comprising a disruption in a PKDL2 gene, wherein the disruption is homozygous, the mouse does not produce functional PKDL2 protein, and exhibits phenotype for increased activity, relative to a wild type mouse. The claims are further drawn to a cell or a tissue isolated from said transgenic mouse, and a method of producing said transgenic mouse.

Breadth of claims and amount of guidance in the specification and working Examples:

In the instant case, claims 25-30 encompass a transgenic mouse that exhibits anti-depressive behavior. The specification does not provide an enabling disclosure for how to use the transgenic mouse as claimed. The specification discloses a PKDL2 transgenic knockout mouse, wherein the homozygous knockout mouse exhibits phenotype of increased activity characterized by an increase in total distance traveled in an open field environment. The specification does not provide specific teaching on how to use these mice with the disclosed phenotype. The specification prophetically teaches that the transgenic mouse can be used to screen drugs or as models for diseases, or screening agents that modulates a phenotype of said mouse. However, the specification fails to teach what type of diseases are the disclosed phenotypes related to. The specification also fails to teach how to use the agent that modulates the phenotype associated with PKDL2 gene disruption. As such, one skilled in the art would not know how to use the transgenic mouse with phenotype of increase activity as a disease model or screen drugs for a specific disease. Moreover, the specification fails to teach how to use a cell or tissue isolated from the transgenic mouse. Therefore, the teaching of the specification is limited.

The state of art and the predictability in the art

The state of art at the time of filing considers generating null mutation of a specific gene in mice and phenotypic behavior resulted from the mutation is unpredictable. Crawley et al. (1997, Psychopharmacology, Vol 132, pages 107-124) teaches that the phenotype of a mutant mouse is not only the result of the targeted gene, but it also reflects interactions with background gene, and other unknown mutations in the genetic background (see pages 107 last paragraph through page 108 1st paragraph). The article further teaches that not all isogenic backgrounds are appropriate for a given study, since the behavioral characteristics of certain isogenic strains could overshadow the effects of the targeted mutations (see page 108, 1st col., lines 10-14). Furthermore, it points out that no single behavior commonly measured in the open field appears to reflect only anxiety or emotional reactivity. Moreover, two strains commonly used in ES cell and knockout generation C57BL/6 and various substrains of 129 are unusual on many standard behavioral paradigms. The unique traits of 129 and C57BL/6 mice are examples of a widespread problem for interpretation of behavioral phenotypes of null mutations, given the genetic diversity that exists amongst the dozens of other commonly available inbred mouse strains (see page 108, 2nd paragraph). Therefore, whether the phenotype of increased activity is result from null mutation alone is unpredictable.

The state of art at the time of the filing is silent on a transgenic mouse whose genome comprises a disruption in an endogenous PKDL2 gene, wherein the disruption is homozygous, said mouse lacks production of the PKDL2 protein, and said mouse exhibits phenotypic feature of increased activity, as compared to a wild type mouse. The art does not provide any teaching regarding the relationship between PKDL2 function and increased activity. The art is also silent on what type of disease is related to PKDL2 dysfunction that would result in the disclosed

phenotype. As such, whether transgenic mouse exhibits phenotype of increased activity can be used for a disease model or screening for drugs is unpredictable. One skilled in the art would have to engage in undue experimentation to use the invention as claimed. Therefore, the claimed invention is not enabled by the instant specification.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Celine X Qian whose telephone number is 571-272-0777. The examiner can normally be reached on 9:30-6:00 M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel Ph.D. can be reached on 571-272-0781. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Celine Qian, Ph.D.

Notice of References Cited	Application/Control No. 10/005,211	Applicant(s)/Patent Under Reexamination ALLEN, KEITH D.04	
	Examiner Celine X Qian	Art Unit 1636	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Crawley et al. (1997, Psychopharmacology, Vol 132, pages 107-124)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Psychopharmacology (1997) 132:107–124

REVIEW

Jacqueline N. Crawley · John K. Belknap
Allan Collins · John C. Crabbe · Wayne Frankel
Norman Henderson · Robert J. Hitzemann
Stephen C. Maxson · Lucinda L. Miner
Alcino J. Silva · Jeanne M. Wehner
Anthony Wynshaw-Boris · Richard Paylor

Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies

Received: 8 November 1996 / Final version: 15 March 1997

Abstract Choosing the best genetic strains of mice for developing a new knockout or transgenic mouse requires extensive knowledge of the endogenous traits of inbred strains. Background genes from the parental strains may interact with the mutated gene, in a manner which could severely compromise the interpretation of the mutant phenotype. The present overview summarizes the literature on a wide variety of behavioral traits for the 129, C57BL/6, DBA/2, and many other inbred strains of mice. Strain distributions are described for open field activity, learning and memory tasks, aggression, sexual and parental behaviors, acoustic startle and prepulse inhibition, and the behavioral actions of ethanol, nicotine, cocaine, opiates, antipsychotics, and anxiolytics. Using the referenced information, molecular geneticists can choose optimal parental strains of mice, and perhaps develop new embryonic stem cell progenitors, for new knockouts and transgenics to investigate gene function, and to serve as animal models in the development of novel therapeutics for human genetic diseases.

Key words Mouse · Inbred strains · Behavior · Genetics · Locomotion · Open field activity · Learning · Memory · Aggression · Parental behaviors · Acoustic startle · Prepulse inhibition · Alcohol · Nicotine · Cocaine · Opiates · Haloperidol · Diazepam · Breeding · Embryonic stem cell lines · Transgenic · Knockouts · Null mutation

J.N. Crawley (✉) · R. Paylor
Section on Behavioral Neuropharmacology,
Experimental Therapeutics Branch,
National Institute of Mental Health, Building 10, Room 4D11,
Bethesda, MD 20892-1375, USA

J.K. Belknap · J.C. Crabbe
Portland Alcohol Research Center,
Department of Behavioral Neuroscience,
Oregon Health Sciences University and VA Medical Center,
Portland, OR 97201, USA

A. Collins · J.M. Wehner
Institute for Behavioral Genetics, University of Colorado,
Boulder, CO 80303, USA

W. Frankel
The Jackson Laboratory, Bar Harbor, ME 04609, USA

N. Henderson
Department of Psychology, Oberlin College,
Oberlin, OH 44074-1086, USA

R.J. Hitzemann
Department of Psychiatry, State University of New York,
Stony Brook, NY 11794–8101, USA

S.C. Maxson
Biobehavioral Sciences Graduate Degree Program and Department
of Psychology, University of Connecticut,
Storrs, CT 06269-4154, USA

L.L. Miner
Molecular Neurobiology Laboratory,
National Institute on Drug Abuse, Baltimore, MD 21224, USA

A.J. Silva
Cold Spring Harbor Laboratory,
Cold Spring Harbor, NY 11724, USA

A. Wynshaw-Boris
Laboratory of Genetic Disease Research,
National Institute, Human Genome Research Institute,
Bethesda, MD 20892-4470, USA

Introduction

Recent advances in molecular genetics have greatly expanded the search for genetic determinants of complex behaviors (Lander and Schork 1994). Transgenic and knockout mice provide a powerful new tool for the elucidation of biological functions. Molecular geneticists have been spectacularly successful in developing the technology for targeted germ line mutations. A great many new knockouts have been made for genes expressed in the mammalian central nervous system. Behavioral neuroscientists are now analyzing the behavioral phenotypes of these fascinating mice.

The phenotype of a mutant mouse is not only the result of the targeted gene, but it also reflects interactions

with background genes, and other unknown mutations in the genetic background. Proteins do not work alone but in large biochemical complexes and cascades, where each step is directly dependent on many other simultaneous molecular events. Thus, genetic background should be as carefully controlled as any other experimental variable. The simplest way to do this is to derive and maintain mutations in an isogenic genetic background, a standard practice in other model organisms such as yeast, *Drosophila*, and *C. elegans*. However, not all isogenic backgrounds are appropriate for a given study, since the behavioral characteristics of certain isogenic strains could overshadow the effects of the targeted mutation. Understanding of the behavioral phenotype of the strain in which a mutation will be studied can avoid overinterpretation of the mutant phenotype.

Since natural strain differences exist for behavioral traits, the genetic background of the inbred mouse strains must be carefully considered in the interpretation of behavioral phenotypes of knockout mice (Crawley 1996; Gerlai 1996). Embryonic stem cells from substrains of 129 mice are commonly used in targeting experiments. C57BL/6 is the strain commonly used for breeding, and as the background strain for spontaneous mutations. However, mice from the various substrains of 129 and C57BL/6 are unusual on many standard behavioral paradigms. For example, 129/Sv mice are impaired on many learning tasks (described below). The allelic contributions from each parental strain can have profound interactive effects with the mutated gene of interest. The unique traits of 129 and C57BL/6 mice are examples of a widespread problem for the interpretation of behavioral phenotypes of null mutations, given the genetic diversity that exists amongst the dozens of other commonly available inbred mouse strains.

A comprehensive database on behavioral phenotypes of inbred strains of mice would provide the information needed by molecular geneticists to make the optimal choice of parental strains and breeding strategies for the expected phenotype of each targeted mutation, and to interpret the results appropriately. Towards this goal, a guide to some of the critical literature on strain distributions for a variety of commonly used behavioral paradigms is assembled herein. The findings described below summarize the behavioral genetics literature and refer the reader to pertinent reviews. The 129 substrains and the C57BL substrains are emphasized, including summaries assembled by the present authors at a recent meeting of behavioral geneticists, behavioral neuroscientists, and molecular geneticists (Workshop on Behavioral Phenotypes of Inbred Strains of Mice 1996). This first step towards conveying the rich diversity of inbred mouse strains available to the molecular genetics community may also highlight the need for thorough behavioral testing of 129 substrains, and for further development and testing of new embryonic stem cell progenitor lines.

Strain distributions of spontaneous behaviors

Open field locomotion

Motor activity underlies almost every mouse behavioral paradigm. Dysfunctions in physical movement can produce false positives and false negatives on behaviors of interest for knockout and transgenic mice. Simple, automated tests of spontaneous locomotion are routinely performed on homozygous mutants, heterozygotes, and wild-type littermates, before any further behavioral testing begins. Photocell beam measurements of open field locomotion, in standard photocell-equipped automated open field equipment, can evaluate total amount of movement, rate of movement, and type of spontaneous activity, over a 5- to 60-min test period.

The open field test is also one of the oldest, most extensively used, and simplest measures of mouse and rat emotional behavior. Over 60 years ago, Hall provided the rationale for using decreased ambulation and increased defecation in a brightly lit open field as indices of heightened emotionality (Hall 1934, 1936). Subsequently, over 20 additional behavioral measures have been proposed as indices of emotionality/anxiety in the open field, as well as suggested sequential analyses of multiple behaviors (Bindra and Thompson 1953; Archer 1973; Walsh and Cummins 1976; Hay 1985). Of these additional measures, rearing behavior, which decreases in an anxiogenic environment, and thigmotaxis, the proportion of time the animal remains close to the walls of the open field, are the additional behaviors most commonly assessed. The availability of open field apparatus which can record gross activity in three dimensions allows complete automation of activity scoring. High levels of ambulation and rearing are positively correlated with each other and negatively correlated with defecation (Henderson 1967; Van Abeelen 1977; De Fries et al. 1978). Increasing the stressful properties of the open field, by increasing illumination level or background noise, generally results in decreased activity. Environmental conditions and prior treatments, such as handling, stress, surgery, and drug treatments, will affect performance on open field testing.

No single behavior commonly measured in the open field appears to reflect only anxiety or emotional reactivity. The open field parameters all appear to reflect multiple underlying traits. Thus, genes linked to open field performance may be involved in the regulation of general locomotor activity, exploratory activity, olfaction and vision, as well as fear and anxiety. In low stress test environments, general activity probably dominates observed score variance, whereas in stressful test environments, e.g., high levels of illumination which are stressful for nocturnal rodents, anxiety-based factors are likely to be a large component of observed variance in activity. Newer behavioral models which are more specific for anxiety are discussed below, in the section on anxiolytics.

Reviews using several types of open field equipment and test conditions have described strain distributions of

inbred mice on open field activity (Crabbe 1986; DeFries et al. 1978; Henderson 1986; Marks et al. 1989a; Mathis et al. 1994). There are many different types of apparatus for measuring open field behavior, and for measuring other types of spontaneous activity, and strain distributions vary with type of test and equipment. In general, the C57 inbred strains of mice, including C57BL/6, C57BL/10, C57BR, and C57L, consistently show high levels of open field locomotion and low levels of anxiety-related measures in the open field. Intermediate strains include the DBA/2, CBA, AKR, and LP. Strains typically exhibiting low locomotor activity and high levels of emotional reactivity include DBA/1, BALB/c and A/J. Other strains show greater inconsistencies across studies. In general, albino strains are overrepresented at the high anxiety end of the distribution. In part, this may be a function of the added stress of high illumination to albino mice. Recent quantitative trait loci analyses (QTL) have identified six chromosomal loci which are strongly linked to open field activity (Flint et al. 1995). Three significant QTLs accounted for most of the genetic variance in open field activity and defecation, and did not show linkage to a separate measure of general activity, closed arm entries in the elevated plus maze, suggesting that sites on chromosomes 1, 12, and 15 may represent facets of the genetic basis of emotionality.

The best choice of an inbred background on which to explore the impact of a null mutation on locomotor activity and emotional reactivity can be taken from these referenced strain distributions. Highly active strains are the best choice when the mutation is predicted to decrease locomotion or increase anxiety; low activity strains are the best choice when the mutation is predicted to increase activity (e.g. hyperlocomotion predicted by the dopamine transporter knockout; Giros et al. 1996).

Learning and memory

Mouse inbred strain differences for performance on learning and memory tasks are well documented. A wide variety of paradigms have been examined which measure complex learning, simple associative learning and avoidance learning. Further, the important distinction between true learning differences versus sensory impairments that lead to poor performance is evaluated for inbred mouse strains. Visual acuity is important for spatial learning tasks. Some mouse strains are albino and demonstrate poor vision under bright lights, while others have the retinal degeneration gene which leads to blindness in adult mice. Auditory function is important for paradigms involving conditioning to the presentation of a tone; some strains show deafness as a function of age. Responses to electrical shock may also vary across strains; jump thresholds should be determined in those paradigms which employ such aversive stimuli. Analogously, the role of strain differences in motivation, whether appetitive or aversive, needs to be rigorously dissociated from true learning and memory differences.

Complex learning tasks

These are behavioral tasks which require an animal to use multiple pieces of information simultaneously to learn. The Morris water task is frequently used to examine spatial learning in rats (Morris 1981), and has been modified slightly for use in mice (Owen et al. 1997; Upchurch and Wehner 1989). Animals are trained to locate a hidden platform in a circular pool filled with opaque water, using distal room cues. Latencies to locate the platform are recorded on each trial, but in mice do not provide robust measures of spatial learning. Measurements of spatial learning require analysis of spatial selectivity by examining performance on a probe trial, in which the platform is removed and the search pattern of the mouse is evaluated. An animal that has learned the position of the platform will demonstrate greater platform crosses over the trained site versus other possible sites, and spend more time in the area of the pool which previously contained the platform versus other areas of the pool. Visual acuity is examined by measuring the latencies to locate a visibly marked platform that is moved to various positions throughout training. The spatial selectivity of inbred mouse strains and F1 hybrids on Morris water task performance is shown in Table 1.

In another form of complex learning, contextual fear-conditioning, animals are trained to associate a shock paired with a tone, in a particular contextual environment. The methods of Fanselow (1990) and LeDoux (Phillips and LeDoux 1992) were adapted to mice by Paylor et al. (1994). Bouts of behavioral immobility, termed "freezing", are used as a measure of performance. Adequate performance is defined as learning to discriminate a pairing of shock and tone in a particular context versus an altered context. The performance of inbred strains and F1 hybrids on contextual fear-conditioning is shown in Table 1.

Spatial and/or working memory has been examined in eight-way radial arm maze tasks, in which animals must remember the arm of a maze in which they previously obtained a food reward. Rank orders of performance as defined by the number of correct choices using appetitive rewards have been determined for several inbred mouse strains (Ammassari-Teule et al. 1985, 1993; Reinstein et al. 1983). "Good" and "poor" strains are defined in Table 1. Conditional spatial alternation in a water filled T-maze is also sensitive for detection of strain differences, as shown in Table 1. In contrast, a spatial open-field test (Roullet and Lassalle 1990) did not allow detection of significant differences across genotypes, when nine inbred strains and three F1 hybrids were examined, because variation within individual strains was great.

Avoidance tasks

In avoidance paradigms, animals do not enter or will quickly leave a location where they previously received a footshock. One-way avoidance tasks can require either

Table 1 Performance of inbred mouse strains and F1 hybrids for complex learning

Good	Poor	Visually impaired
A. Morris water task[a]		
C57BL/6J	129Sv/J	A/J
C57BL/10J	DBA/2	SJL/J
129/SvevTacfBr	LP/J	C3H/Ibg
BALB129F1	BALB/cByJ	FVB/NJ
B6D2F1		Bub/BNJ
B10C3F1		
129B6F1		
B. Contextual fear conditioning[b]		
C57BL/6J	FVB/NJ	
C57BL/10J	DBA/2	
129/SvJ	Bub/BNJ	
129/SvevTacfBr	C3H/Ibg	
SJL/J		
BALB/cByJ		
LP/J		
BALB129F1		
FVB129F1		
B6D2F1		
B6SJLF1		
B10C3F1		
129B6F1		
C. Eight-way radial arm maze[c]		
C57BL/6	NZB	
DBA/2	CBA	
CB6F1	C3H/He	
B6D2F1	BALB/C	
D. Conditional spatial alternation[d]		
C57BL/6Ibg	DBA/2Ibg	

[a] Based on data and conclusions from Owen et al. (1997) and Upchurch and Wehner (1989), in which good-learning strains showed significantly greater crosses at the trained platform site compared to three other sites during a probe trial after 36 training trials. Poor-learning strains did not show greater crosses over the trained site compared to other sites. Visually impaired animals could not reliably locate a visibly marked platform in less than 30 s after eight trials

[b] Based on data and conclusions from Owen et al. (1997). Good contextual learners were those strains that exhibited greater freezing in the training context than in the altered context as measured by bouts of freezing during a defined period of time. Poor learning strains did not show greater freezing in the context versus the altered context

[c] Based on data and conclusions from Ammassari-Teule et al. (1993), in which strains were compared on the number of correct, i.e., unrepeated arm, choices in a fully baited eight-way radial arm maze and data and conclusions from Roullet and Lassalle (1995), in which strains were compared for the number of errors in an eight-way baited radial arm maze over five training sessions. No performance criterion was established but good learning strains were significantly better than poor learning strains in both studies

[d] Based on data and conclusions from Paylor et al. (1993), in which nine out of ten correct trials were used as the criterion of learning. The poor learning strain required at least twice the number of training trials

an active (moving) or passive (resting) response, and performance must evaluate the possible role of general activity (see previous section). Two-way and avoidance-avoidance paradigms introduce a potentially confounding contribution of anxiety-related behaviors (see below). Literature published before 1972 was previously reviewed and evaluated by Wahlsten (1972). Some of the more recent literature is summarized in Table 2.

Table 2 Performance of inbred mouse strains and F1 hybrids on avoidance learning

A. One-way avoidance[a]
5 days of training DBA/2 >C57BL/6 >C3H/HeJ
13 days of training DBA/2=C57BL/6=C3H/HeJ

B. Two-way avoidance[b]
DBA/2 >BALB/CJ >NMRI >C57BL/6J >SM/J >C3H

C. Avoidance – avoidance[c]
CBA/CaJ >BALB/CJ=DBA/1J >SEC/1REJ >RF/J >LP/J >A/J >C57BL/10J

[a] Weinberger et al. (1992)
[b] Buselmaier et al. (1981) and Lipp et al. (1989)
[c] Henderson (1989)

The best choice of an inbred background on which to explore the impact of a null mutation on learning appears to be C57BL/6. C57BL/6 are moderate learners, such that either an impairment or an improvement could theoretically be observed. However, breeding characteristics and the possibility of lethality of a particular null mutation on an inbred background must also be examined for each specific mutation. The data summarized here support the view that many inbred mouse strains perform poorly on complex learning tasks and several also perform poorly on avoidance tasks.

Aggressive behaviors

Here, aggressive behavior is defined for mice as that with the potential for attack bites on another animal, and it is suggested that predation, infanticide, defense, and offense are types of aggressive behaviors in mice (Brain 1979; Maxson 1992a). This section concerns strain differences in offense; the literature on predation, infanticide, and defense is summarized by Maxson (1992a).

Male offense

The first research on strain differences for male mouse offensive behavior was reported more than 50 years ago (Ginsburg and Allee 1942; Scott 1942). Since then, many articles provide extensive information on strain distributions and male offense (Scott 1966; Lagerspetz and Lagerspetz 1974; Simon 1979; Maxson 1981; Hewitt and Broadhurst 1983; Maxson et al. 1983; Michard and Carlier 1985; Jones and Brain 1987; Guillot et al. 1994; Kulikov and Popova 1996). For example, in either homogenous set/novel cage tests or standard opponent/novel cage tests, it has been reported than DBA/1 and DBA/2 are more aggressive (offense) than C57BL/6 and C57BL/10 males (Selmanoff et al. 1976, 1977; Guillot et al. 1994). However, these strain differences and others in offense depend on life history, test situation, and opponent type (Maxson 1992b). For example, they are re-

versed (Ogawa et al. 1996) or eliminated (Jones and Brain 1987) with anosmic standard opponents. Regardless, it would seem to be best to select life history, test situation, and opponent type which give an intermediate level of offense in a strain to be used in knockout research. This would permit detection of increases or decreases in offense as a consequence of the knockout mutation. Because the control animals had a low level of offense, the studies with 5-HT_{1B} (Saudou et al. 1994), MAO-A (Cases et al. 1995), and NosI (Nelson et al. 1995) knockouts could only have detected an incremental effect on the mutant.

Female offense

Female aggression, which may be of the offense type, is dependent on reproductive state. Offense in female mice is assessed in three reproductive conditions: neither pregnant or lactating, pregnant but not lactating, or lactating but not pregnant. Strain differences in offense for one or more of these reproductive states have been described for DBA/2, C57BL/6, C57BL/10, AKR, BALB/c, C3H, CBA, and NZW inbred strains (Ogawa and Makino 1981, 1984; Broida and Svare 1982; Jones and Brain 1987; Svare 1988). Again, strain differences for female offense depend on life history, test situation, and type of opponent. For example, non-pregnant and non-lactating DBA/2 and C57BL/6 females do not differ in offense against an intruder male (Ogawa and Makino 1981, 1984), whereas C57BL/6 females are more aggressive (offense) than DBA/2 females against a lactating intruder female (Haug et al. 1992). Similarly, there is a difference in offense between DBA/2 and C57BL/6 females for an anosmic R-S male intruder (Svare 1988) but not for an anosmic TO male intruder (Jones and Brain 1987). Regardless, it would be best, as with male offense, to select life history, test situation, and opponent type which give an intermediate level of offense in females for the strain to be used for mutant testing.

No general recommendation can be given for a single best strain for transgenic and knockout studies of aggressive behaviors, since aggressive behavior depends on many developmental and experiential factors. The strain, test paradigm, and experience should be selected such that increases and decreases in an aggressive behavior can be detected for the null mutation.

Reproductive behaviors

Sexual behaviors

The focus for strain differences in sexual behaviors has primarily been on male copulatory mounts, intromissions, and ejaculations. Latency, frequency, duration, and other measures have been used as indices for these behaviors (McGill 1962, 1970). The stimulus female is usually artificially brought into estrus with hormone treatment. Male copulatory behaviors have been described for DBA/2, C57BL, C57BL/6, BALB/c, AKR, A/J, C3H, and CBA inbred strains of mice (McGill 1962; McGill and Ransom 1968; Vale and Ray 1972; Mosig and Dewsbury 1976; Batty 1978; Ogawa et al. 1996, 1997). High copulatory behaviors were reported for C57BL and C57BL/6, lower copulatory behaviors were reported for DBA/2 and AKR, and the strains BALB/c and A/J showed the lowest copulatory behaviors. The same strain differences were obtained for the most part with estrus females of different strains or F1s.

Parental behaviors

Parental behaviors include pup retrieval, nesting with pup, nursing of pup, and licking pup. The most extensive strain distributions for maternal pup care are by Carlier et al. (1982) and Cohen-Salmon et al. (1982, 1985). When mothers and infants are of the same strain, then CBA/H, C4H/Ico, C57BL/6, and CBA/J are better pup retrievers than BALB/c, NZB, DBA/2, XLII, A/J and AKR. These data may be useful in selecting strains for knockout research on maternal behaviors where the wild type or knockout mother is paired with pups of the wild type. For example, DBA/2 mothers have intermediate values for pup retrieving and pup nesting, and they may therefore be a good background strain to detect incremental or decremental effects of knockout mutants. It must be recognized that such differences may be due to pup as well as to mother's strain (Cohen-Salmon et al. 1985).

Acoustic startle and prepulse inhibition

Acoustic startle is the reflex response to a sudden, loud noise. Prepulse inhibition (PPI) is the suppression of the normal response to a startling stimulus when that stimulus is immediately preceded by a weak prestimulus or prepulse (Graham 1975). In the standard paradigm, the startle response is measured by presenting a loud sound (acoustic startle stimulus) or puff of air (tactile startle stimulus) to a subject and measuring the reflexive startle response. A low level of acoustic stimulus that itself does not evoke a startle response is then presented, less than 100 ms before the startle stimulus, and the reflexive response to the startle stimulus is measured, using eye blink in humans and muscle twitch in rodents. The reduction in the startle response when the same startle stimulus is immediately preceded by the weak prepulse is used as the measure of prepulse inhibition.

A number of studies have shown that schizophrenic patients have an impaired prepulse inhibition response (Braff et al. 1978; Franks et al. 1983; Freedman et al. 1983). The impairment observed in schizophrenics is thought to reflect an underlying problem with inhibitory mechanisms similar to those used for sensorimotor gating (Braff et al. 1978; Freedman et al. 1987). Rats and

Table 3 Startle response of 11 inbred strains of mice

Tactile stimulus*		Acoustic stimulus*	
Inbred strain	Startle response mean (±SEM)	Inbred strain	Startle response mean (±SEM)
C57BL/10J	3292 (±233)	C57BL/10J	2417 (±283)
FVB/NJ	2182 (±219)	FVB/NJ	2009 (±163)
BALB/cByJ	1623 (±173)	BALB/cByJ	1970 (±109)
C3H/HeJ	1601 (±110)	C57BL/6J	1317 (±121)
129/SvEvTac	1407 (±261)	A/J	1237 (±193)
C57BL/6J	1306 (±140)	129/SvEvTac	1139 (±153)
AKR/J	1187 (±100)	AKR/J	892 (±97)
A/J	1038 (±167)	C3H/HeJ	888 (±90)
DBA/2J	705 (±138)	DBA/2J	440 (±91)
129/J	569 (±148)	129/SvJ	186 (±33)
129/SvJ	425 (±85)	129/J	181 (±47)

* The maximum startle response (arbitrary units) to a tactile stimulus (40 ms, 12 psi airpuff) and an acoustic stimulus (40 ms, 120 dB sound burst) for male mice from 11 inbred strains. Mice were first tested using the tactile stimulus followed a week later with the acoustic stimulus. Adapted from Paylor and Crawley (1997)

Table 4 Prepulse inhibition of 11 inbred strains of mice

90 dB prepulse inhibition of the tactile startle response*		90 dB prepulse inhibition of the acoustic startle response*	
Inbred strain	% PPI mean (±SEM)	Inbred strain	% PPI mean (±SEM)
129/SvEvTac	79 (±3)	129/SvEvTac	87 (±2)
AKR/J	72 (±3)	AKR/J	84 (±3)
129/SvJ	70 (±5)	129/SvJ	66 (±7)
A/J	66 (±3)	129/J	66 (±7)
129/J	65 (±15)	DBA/2J	56 (±4)
DBA/2J	48 (±9)	FVB/NJ	64 (±5)
FVB/NJ	39 (±6)	A/J	61 (±7)
BALB/cByJ	35 (±6)	BALB/cByJ	51 (±7)
C3H/HeJ	32 (±7)	C3H/HeJ	48 (±6)
C57BL/10J	31 (±3)	C57BL/10J	47 (±2)
C57BL/6J	31 (±8)	C57BL/6J	36 (±3)

* The %PPI of the tactile and acoustic startle responses for male mice from 11 inbred strains. A range (74–90 dB) of acoustic prepulse sound levels was presented 100 ms before either the tactile or acoustic startle stimuli. The table shows the %PPI using the 90 dB prepulse stimulus. Adapted from Paylor and Crawley (1997)

mice show schizophrenic-like reductions in prepulse inhibition when treated with dopaminergic agonists (Geyer et al. 1990; Swerdlow et al. 1991, 1994a, b; Swerdlow and Geyer 1993; Johansson et al. 1995). The prepulse inhibition paradigm has quickly become an ideal animal model to study the mechanisms underlying the sensorimotor gating deficit observed in schizophrenia (Geyer et al. 1990; Swerdlow et al. 1994a) and for screening new antipsychotic therapeutics.

Startle and prepulse inhibition have been characterized in 11 inbred strains of mice (Paylor and Crawley 1997). Male mice (55–80 days old) from Jackson Laboratories (Bar Harbor, Maine, USA) or Taconic Farms (Germantown, N.Y., USA) were tested for amount of prepulse inhibition of a tactile and acoustic startle response using the SR-Lab System (San Diego Instruments). Table 3 shows that different inbred strains of mice varied on both acoustic and tactile startle, with the C57BL/10J showing the largest response and the 129/J and 129/SvJ showing the smallest response. Acoustic and tactile startle showed similar strain distributions. Interestingly, the 10J and 6J, which are substrains of the C57BL strain, show different levels of startle. Similarly, there is considerable difference in the amount of startle among the 129/J and 129/SvJ strains compared to the 129/SvEvTac strain. Because the number of genes polymorphic between such closely-related substrains is relatively few, such a pattern is suggestive of a trait controlled by only a few genes.

Table 4 illustrates the differences in prepulse inhibition among inbred strains, with the 129/SvEvTac and the AKR/J strains showing the most PPI while the C57BL/6J and 10J showed some of the lowest levels of PPI. Strain distributions were similar for PPI of acoustic and tactile startle; however, they are somewhat different using lower prepulse sound levels. Interestingly, there were no significant correlations in strain distributions for startle versus prepulse inhibition, suggesting that the genetic substrates for startle and PPI behaviors are very different.

Additional strain distributions have been published for acoustic startle (Marks et al. 1989b) and for prepulse inhibition (Willott et al. 1994; Bullock et al. 1995), using different parameters and equipment.

Recommendations of inbred strains with good startle responses include C57BL/10J, FVB/NJ, and BALB/cByJ. These can be used to detect behavioral phenotypes of null mutations predicted to impair startle. Recommendations of inbred strains with good prepulse inhibition include 129/SvEvTac an AKR/J. These can be used to detect behavioral phenotypes of null mutations predicted to impair prepulse inhibition. Strains with poor prepulse inhibition, including C57BL/6J and C57BL/10J, can be used to investigate mutations postulated to improve prepulse inhibition.

Strain distributions of drug-induced behaviors

Ethanol

Genetic determinants of the behavioral actions of ethanol provide an excellent example of the strengths and caveats of behavioral genetics analyses. The principles described herein are relevant to many other drug-induced behaviors described in subsequent sections.

The sedative hypnotic ethyl alcohol elicits a wide variety of behavioral responses in laboratory mice. Low to moderate doses produce behavioral excitation (e.g., increased locomotor activity and exploration, and increases in behaviors interpreted as reflecting anxiolysis, such as emergence from a dark compartment). It is curious that low doses given to rats seem to *reduce* locomotor activity. Moderate doses are anticonvulsant, and elicit a spectrum of responses, most of which are generally referred to as "intoxication," including disturbances in thermoregulation, loss of balance and ataxia, and reduced muscle tone. High doses suppress motor reflexes such as the righting reflex, reduce locomotor activity and exploration, induce amnesia, and, several hours later, reveal a rebound nervous system hyperexcitable state (proconvulsant effect, i.e. reduced threshold sensitivity to seizures) that defines a state of acute dependence and withdrawal. Lethal doses suppress respiratory function.

In addition to these dose-related (and frequently biphasic) responses, ethanol has reinforcing effects. Some strains ingest ethanol solutions by choice and will work to obtain access. Low doses can serve as an interoceptive cue that will sustain a conditioned preference for the location where ethanol was given. It is curious that in rats, the ethanol cue induces a conditioned place *aversion*. Somewhat higher doses in mice (and rats) can serve to elicit a conditioned aversion to a flavor paired with drug administration. Finally, when ethanol is chronically administered, most sedative responses will show reduced magnitude (tolerance), but some stimulant responses (notably, locomotor increases) show enhancement (sensitization). Withdrawal severity becomes more pronounced with higher doses, longer durations of exposure, and repeated cycles of administration.

Inbred strain differences

With this background, it is perhaps not surprising that it is not possible to identify a single inbred strain that is "sensitive," nor one that is "resistant," to ethanol. Strains differ in response to ethanol on every response parameter just described, but the same strains are not sensitive to all ethanol effects. Where multiple strains have been examined for a single response in carefully controlled environmental conditions, the pattern of strain differences for a single response may also vary by dose of ethanol. For example, in one study, sensitivity and tolerance to the hypothermic effects of ethanol at 2, 3, or 4 g/kg was determined in C57BL/6J and DBA/2J inbred strains and 19 of the recombinant inbred (RI) strains rederived from their F_2 cross (Crabbe et al. 1994a). In this set of strains, each strain is homozygous at each gene, and there are not more than two alleles possible (originating from either the C57BL/6J or the DBA/2J progenitor, for genes where they possess different alleles). Despite the simplicity of this genetic system, mean strain initial sensitivity to one dose of ethanol was not perfectly correlated with mean strain sensitivity to the other doses. This means that it is not possible to assign an unequivocal strain rank-order for "ethanol hypothermic sensitivity."

A second feature of the genetic control of sensitivity to a particular effect of ethanol is revealed by systematic screening of a panel of 15–20 inbred strains. Observer-scored ataxia in the home cage (Crabbe et al. 1982), ability to retain balance on a stationary dowel (Crabbe et al. 1982) or on a rotating rod (Gallaher et al. 1996), and locomotor ataxia in the grid test (Phillips et al. 1996) all seem deceptively similar from the point of view of the human experimenter. However, systematic data suggest that from the mouse's point of view, they appear to be rather different problems. In general, the genetic correlations among strain sensitivities to these ethanol effects were not substantial (Crabbe et al. 1996). The influence of the genome seems rather finely tuned to a particular ethanol response, even when several such responses appear to fall within the physiological domain, "ataxia." Characterizing a strain as "sensitive to ethanol-induced ataxia" requires several corrobating tests, of which the rotarod is one. By similar reasoning, interpreting a deficit in performance on the Morris water maze by a mouse bearing a gene knockout as evidence of "reduced hippocampal function" requires supporting data from a number of tasks with demonstrated hippocampal dependence. Furthermore, the above studies employed only a single dose of ethanol for each task, so each task-specific pattern of strain differences could change if a different dose of ethanol were used, of if a different time window after injection were examined.

Are there any "generalizable" strain differences in ethanol responsiveness?

The reader should not be too discouraged by the apparent specificity of genetic influence on behavioral endpoints in mice. Certain generalizations appear to be relatively robust against procedural variations such as ethanol dose, time window, and other details of the method employed. A systematic review of the inbred strain literature on responses to alcohol and other drugs appeared nearly 20 years ago (Broadhurst 1978). In a review concentrating on alcohol responses (Belknap 1980), results on fewer than 25 studies, which constituted most of the relatively few studies that were available, suggested that there were sufficient data for comparing only three inbred strains of mice – C57BL/6J, DBA/2J, and BALB/cJ. Excellent subsequent reviews of this literature have since appeared (e.g., Deitrich and Spuhler 1984). Horowitz and Dudek (1983) pointed out the response-specificity of genetic sensitivity to drugs. They also offer instructive discussion of the many intervening variables that can confound simple attribution of an individual's characteristic drug sensitivity to that individual's genotype. For example, if strains differ in drug absorption or metabolism, administering an equal dose to each strain may lead to different brain concentrations of the drug at the time of testing. A strain with low brain drug levels may be

deemed "insensitive," but the mechanisms underlying this would be pharmacokinetic and not due to differences in the sensitivity of the brain systems mediating drug response. This concern appears not to be critical for many responses to ethanol. When sensitivity of 15 inbred strains to several ethanol responses was screened, blood or brain ethanol concentration was not significantly correlated with behavioral sensitivity for any variable (Crabbe et al. 1994b).

A recent review of mouse inbred strain difference studies employing ethanol summarized results from over 100 studies (Phillips and Crabbe 1991). The consistent finding is that C57BL/6J mice (and other related C57 strains) willingly ingest ethanol solutions, while DBA/2J (and other, related DBA strains) avoid any detectable concentrations of ethanol (McClearn and Rodgers 1959; Fuller 1964; Schneider et al. 1973; Phillips and Crabbe 1991; Belknap and O'Toole, 1991; Belknap et al. 1993a, b). The DBA/2 strain is, however, generally more susceptible to the low-dose locomotor stimulant effects of ethanol than the C57BL/6 strain. The relative sensitivity to ethanol of the C57BL/2 and DBA/2 strains depends upon the response variable studied: neither appears to be systematically more sensitive across all other response domains.

The severity of physical dependence, as evidenced by withdrawal symptoms following cessation of ethanol administration, is markedly more severe in DBA/2 strains than in C57BL/6 strains, and DBA/2 strains are generally the most severely withdrawing strains compared to other inbred strains as well (Crabbe et al. 1983; Metten and Crabbe 1994). This generalization cannot be extended to ethanol tolerance, where the strain difference depends upon the particular response studied.

A final issue where inbred strain studies appear to offer firm support over the years is the genetic relationship between sensitivity and tolerance to ethanol. An early survey of 20 standard inbred strains found that sensitivity and tolerance to ethanol hypothermia were genetically correlated (Crabbe et al. 1982). A more recent survey of more than 20 recombinant inbred strains corroborated this result (Crabbe et al. 1994a), and found that sensitivity and tolerance to rotarod ataxia (Gallaher et al. 1996) and grid-test ataxia (Phillips et al. 1996) also showed a positive correlation between magnitude of sensitivity and tolerance. Comparison of RI strain sensitivities across these three measures, however, revealed no substantial correlations among the three sensitivity measures, or among the three tolerance measures (Crabbe et al. 1996). This suggests that a strain (and by extension, an individual mouse) initially predisposed genetically to be sensitive to a particular effect of ethanol is likely to develop more tolerance to that effect with repeated exposure.

The literature on inbred strain differences in response to ethanol argues strongly for not attempting to extrapolate genetic "sensitivity" beyond the specific task studied. That is, the genetic determinants of behavioral sensitivity to one domain of ethanol effects are to a great extent unrelated to those determining other responses. Although strains also differ in blood and brain ethanol concentrations achieved after a fixed dose, studies thus far suggest that strain differences in response to ethanol are more likely to be due to functional differences at the relevant central nervous system target of ethanol. Certain inbred strains have robust and predictable responses to particular ethanol effects, and individual sensitivity, at least to some sedative effects of ethanol, appears to predict propensity to develop tolerance.

Thus, the choice of an "ideal" strain for a particular ethanol response depends upon the response, and whether a high-, moderate- or low-scoring strain is sought.

Nicotine

Current evidence argues, as is the case for alcohol, that genetic factors influence virtually every aspect of nicotine's actions. Inbred mouse strains differ on both the stimulant and depressant effects of a first dose of nicotine, and on the development of tolerance to repeated doses of nicotine.

Table 5 shows the nicotine dose required to produce a standard effect, such as an ED_{50} value, for 19 strains of mice, on a variety of behavioral and physiological measures, using methods previously described (Marks et al. 1989a; Miner and Collins 1989). A principal component analysis of genetic influences on the first dose sensitivity presented in the data of Table 5 detected two major components, one that is typified by nicotine-induced seizures and the other by Y-maze crossing and rearing activity and body temperature (Marks et al. 1989b). One gene which may be common to one of the principal components is the α_4 nicotinic receptor gene, which codes for [^{3}H]-nicotine binding. Approximately 35–40% of the variance in sensitivity to nicotine on the Y-maze and body temperature measures seems to be attributable to differences in the number of [^{3}H]-nicotine binding sites. Similarly, the α_7 nicotinic receptor gene seems to be associated with nicotinic-induced seizures.

Inbred mouse strains also differ on the development of tolerance to nicotine, as measured by the Y-maze and body temperature measures (Marks et al. 1991). Intravenous infusion of saline or a nicotine dose ranging between 0.5 and 6 mg/kg per hour for 16 days produced a right shift in the dose-response curve for the effects of nicotine. The threshold tolerance dose for six inbred strains is given in Table 6. Inbred mouse strains also differ in oral self-administration of nicotine (Robinson et al. 1996). Daily intake of nicotine and water was tested in a two-choice paradigm, using concentrations of nicotine ranging between 10 and 200 μg/ml. As the nicotine concentration increased, all of the strains showed a concentration-dependent decrease in the maximum volume of nicotine-containing solution consumed. The nicotine concentration that produced a 50% decrease in the volume of nicotine solution consumed, the IC_{50} value, is given for six strains in Table 6. Correlations between the preference measures and acute sensitivity to nicotine and

Table 5 Genetic influences on first dose sensitivity to nicotine*

Strain	Respiratory rate (ED_{260})	Startle response (slope)	Y-maze crosses (ED_{50})	Y-maze rears (ED_{50})	Heart rate (ED_{-100})	Body temperature ($ED_{-2°}$)	Nicotine seizures (ED_{50})
A/J/Ibg	0.78±0.09	−1.67±0.61	0.80±0.31	0.41±0.21	0.82±0.13	0.55±0.06	3.12±0.13
AKR/J	1.48±0.09	−0.23±0.46	1.42±0.31	1.26±0.27	1.60±0.25	1.37±0.20	4.95±0.14
BALB/cByJ	0.67±0.17	+2.04±1.48	1.06±0.06	0.97±0.20	0.95±0.33	0.92±0.17	3.79±0.11
BUB/BnJ	1.29±0.23	+2.27±1.75	1.89±0.33	1.32±0.36	1.48±0.24	2.53±0.08	4.52±0.06
CBA/J	0.73±0.31	+2.66±0.94	1.43±0.21	1.41±0.21	1.41±0.19	1.56±0.36	3.53±0.09
C3H/2Ibg	1.10±0.14	+3.70±0.73	1.78±0.33	1.50±0.10	1.25±0.24	1.32±0.09	3.13±0.09
C57BL/6J/Ibg	0.95±0.19	−1.77±1.05	0.51±0.18	0.45±0.18	0.90±0.23	0.80±0.16	5.30±0.26
C57BL/10JU	1.14±0.17	+0.73±1.05	0.49±0.21	0.37±0.27	1.12±0.12	0.61±0.21	3.55±0.04
C57BR/cdJ	0.43±0.24	−0.76±0.22	1.07±0.13	0.92±0.11	1.40±0.20	1.59±0.32	4.62±0.01
C57L/J	0.97±0.47	−0.10±0.06	1.17±0.27	0.80±0.12	1.36±0.40	1.20±0.11	4.99±0.05
C58/J	2.66±0.41	+0.49±0.94	1.82±0.08	1.54±0.22	1.28±0.48	2.07±0.06	5.89±0.19
DBA/1J	1.49±0.11	−0.10±1.32	0.93±0.31	0.94±0.42	0.94±0.24	1.02±0.26	6.16±0.02
DBA/2J/Ibg	1.25±0.11	−0.80±0.87	0.97±0.31	0.80±0.06	0.94±0.24	0.89±0.19	5.21±0.12
LP/J	0.75±0.30	−1.18±0.54	1.04±0.34	0.95±0.26	1.79±0.35	1.30±0.15	4.50±0.01
P/J	0.77±0.23	−0.20±2.30	1.25±0.17	0.96±0.15	1.34±0.23	1.10±0.12	4.30±0.02
RIIIS/J	0.93±0.43	+1.44±0.41	1.62±0.17	1.46±0.17	1.98±0.79	1.19±0.17	3.65±0.14
SJL/J	1.00±0.14	+0.11±0.95	1.32±0.24	1.18±0.22	2.03±0.71	1.23±0.09	4.73±0.24
ST/bJ	0.41±0.04	+4.52±0.94	0.93±0.21	0.64±0.27	0.98±0.18	1.47±0.23	2.34±0.09
SWR/J	1.19±0.25	−0.28±0.46	1.42±0.49	1.19±0.36	2.19±0.45	1.18±0.20	4.48±0.12

* Dose-response curves for each of the responses to nicotine were analyzed by linear regression and parameters reflecting the sensitivity of mice of each of the 19 inbred strains were calculated to provide a comparison of the relative sensitivity of each of the strains to the effects of nicotine. The following calculations were made: respiratory rate, ED_{260}, the dose (mg/kg) required to stimulate respiratory rate to 260 breaths/min; startle response, slope of the dose response curve (change in startle score for each 1 mg/kg increase in dose of nicotine); Y-maze crosses and Y-maze rears, ED_{50}, the dose (mg/kg) required to reduce the number of crosses or rears to 50% of control levels; heart rate, ED_{-100}, the dose (mg/kg) required to reduce heart rate by 100 beats/min; body temperature, $ED_{-2°}$, the dose (mg/kg) required to lower body temperature by 2°; and nicotine-induced seizures, ED_{50}, nicotine dose that elicits seizures in 50% of the animals. Data are presented as the mean±SEM

Table 6 Genetic influences on nicotine tolerance and self-administration*

	Threshold tolerance dose	Nicotine consumption max dose	Nicotine consumption IC_{50}
A	2.32±0.31	5.7±0.3	42.8±8.9
BUB	3.52±0.60	6.2±0.8	72.0±17.2
C3H	3.93±0.44	4.8±0.5	40.2±7.6
C57BL/6	1.12±0.46	11.7±1.1	114.1±20.2
DBA/2	2.73±0.25	8.2±0.6	89.7±12.3
ST/b	-	2.8±0.3	32.4±7.9

* Threshold tolerance dose is reported in terms of mg/kg per hour. Mice of the six strains were infused with nicotine doses ranging between 0.25 and 6.0 mg/kg per hour. The minimal infusion dose that increased the effective dose for nicotine's effects on activity and temperature by 0.25 mg/kg (see Marks et al. 1991) was established as the lowest infusion dose (threshold) that produced reliable tolerance to nicotine. Maximal nicotine consumption (mg/kg per day) and the nicotine concentration (μg/ml) that decreased preference ratios to 50% of the values obtained with a water-μg/ml nicotine solution were calculated as described in Robinson et al. (1996)

threshold tolerance dose were also calculated. The correlation between maximal oral intake dose and ED_{50} for nicotine-induced seizures proved to be highly significant (r=0.89, P<0.01). The higher the nicotine-induced seizure ED_{50} value, the higher the nicotine intake. This finding could mean that a genetic factor related to seizures serves to limit oral intake of nicotine.

Analogous to the ethanol section above, the choice of a "best" strain for a particular nicotine response depends upon the response, and whether a high-, moderate- or low-scoring strain is sought.

Cocaine

Marked inherent differences have been noted for many of the behavioral and physiological effects of cocaine. Among these, the locomotor stimulant property of cocaine has been the most thoroughly characterized (Ruth et al. 1988; deFiebre et al.1989; George 1989; Wiener and Reith 1990; George and Ritz 1991; Jones et al. 1991; Tolliver et al. 1994; Womer et al. 1994; Miner and Marley 1995a; Miner 1997). However, because of the wide variety of techniques used to assess locomotion in these studies (e.g. Y-maze, square and round open-field monitors; differing times of assessment ranging from 5

to 60 min, different strains and substrains tested, and differing doses) comparisons among these studies and therefore a general rank ordering of strain sensitivity is difficult. Nonetheless, close examination of the data can yield some generalities. The C57BL/6 and DBA/2 strains have been the most thoroughly studied and in almost every study, the DBA/2 strain is more sensitive to the locomotor stimulant effects of cocaine than the C57BL/6 strain. Only one study to date has characterized cocaine response in the 129/Sv strain, which along with the C57BL/6 strain, is the most common progenitor strain for knockout mice (Miner 1997). Comparison with the C57BL/6 strain demonstrated that the two strains were nearly identical in locomotor activation after cocaine. A potential confounding factor, however, was that the generally hypoactive 129/Sv strain had a significant three to four-fold increase in activity after saline injection which may be adding to the pharmacological effects of cocaine.

The rewarding effects of cocaine have also been examined in a handful of studies for several inbred mouse strains. Oral cocaine self-administration (George and Goldberg 1989; Seale 1991) has been compared between the C57BL/6 and DBA/2 strains, with the C57BL/6 mice having higher consumption of solutions containing low concentrations of cocaine. The C57BL/6 strain also shows rapid acquisition and maintainance of intravenous cocaine self-administration (Grahame et al. 1995). Conditioned place preference has also been used to compare cocaine reward among several inbred mouse strains (Seale and Carney 1991; Miner 1997). In general, the C57BL/6 and BALB/cBy strains show a behavior pattern indicative of high reward, whereas the DBA/2 does not. Other strains, AKR, C3H, CBA, SJL, show moderate responses. The 129/Sv strain shows no significant reward response using the same conditions that produce significant place preference for the C57BL/6 strain (Miner 1997).

Genetic differences in sensitivity on a variety of other cocaine responses have also been reported for a variety of inbred mouse strains. The phenotypes examined include seizure suseptibility (deFiebre et al. 1989; Marley et al. 1991; Miner and Marley 1995b), kindling (Marley et al. 1991), cardiovascular function (Ruth et al. 1988; deFiebre et al. 1989), sensitization/tolerance (Shuster et al. 1977; Tolliver and Carney 1994; Tolliver et al. 1994) hepatotoxicity (Thompson et al. 1984; Shuster et al. 1988) and thermoregulation (deFiebre et al. 1989). A sizeable data base has been gradually accumulating which describes cocaine sensitivity in a number of inbred mouse strains. To date, the C57BL/6 and DBA/2 strains have been the strains of choice for analysis. While both the C57BL/6 and 129 strains are used in transgenic and knockout studies, the 129 strains have been little studied and their sensitivity to cocaine has been described in only one study (Miner 1997).

While the largest body of data has been obtained from the C57BL/6J strain, given the relatively little work done to date exploring strain differences in cocaine-reluted phenotypes, especially cocaine self-administration, it is too early to recommend an optimal mouse strain for transgenic and knockout studies relevant to cocaine.

Opiates

Morphine and opioid-related traits have been best characterized for the C57BL/6 and DBA/2 strains, and for a recombinant inbred strain, CXBK, derived from C57BL/6By and BALB/cBy. In contrast, the 129 strains have been almost entirely ignored. Most of this literature has been reviewed by Belknap and O'Toole (1991) and Mogil et al. (1996). Following opioid administration, the C57BL/6 strain has distinguished itself as a marked "runner" (high locomotor activation) in the open field, low on hot plate-assessed analgesia, very sensitive to naloxone-induced jumping (an index of withdrawal severity) after chronic morphine exposure, and very high on voluntary intake of morphine solutions. In contrast, the DBA/2 strain is frequently reported to be opposite on all of these opioid-sensitive measures. A partial explanation for these marked strain differences is that the C57BL/6 strain is estimated to differ genetically from other inbred strains to a greater degree than is usually the case for other pairs of strains (Taylor 1972). Populations derived from these two strains have recently been studied at the QTL level for morphine preference drinking (Berrettini et al. 1994) and analgesia (Belknap et al. 1995). The CXBK strain is unique because it is insensitive to many effects of morphine and other opioids, and appears to be deficient in mu opioid receptors but normal in delta opioid receptors. Given the wealth of data concerning them, and their large strain differences, the C57BL/6 and CXBL are good choices of mouse strains for transgenic and knockout studies relevant to opiates.

Antipsychotics

Catalepsy is defined by maintaining a fixed rearing pattern for 30 s, 15 min after the IP administration of haloperidol (Hitzemann et al. 1991). Hitzemann and coworkers have used haloperidol-induced catalepsy in mice as a model to investigate the genetics of neuroleptic-induced extrapyramidal symptoms. Haloperidol-induced catalepsy shows a 30-fold variation in ED_{50} values (determined according to Dixon et al. 1965) among 40 inbred and recombinant inbred (RI) strains of mice; this range of variation is shared by all typical neuroleptics and is not the result of differences in pharmacokinetic parameters (Kanes et al. 1993; Dains et al. 1996). The most sensitive strains and their respective ED_{50} values are BALB/c (0.3 mg/kg), AKR (0.45 mg/kg) and DBA/2 (0.48 mg/kg). The least sensitive strains are BXD RI #2 (7.9 mg/kg), LP (9.5 mg/kg) and C57L (10 mg/kg). The commonly used C57BL/6 strain has an ED_{50} of 3.8 mg/kg. Recommendations of strains for mutations relevant to neuroleptic-induced catalepsy include these most-sensitive and

least-sensitive strains, appropriate to the hypothesis to be tested.

Kanes et al. (1993) observed that among eight inbred mouse strains there is only two-fold variation in the catalepsy induced by the D_1 receptor antagonist, SCH 23390; the most sensitive strain is C57BL/6 (0.2 mg/kg); the least sensitive strain is AKR (0.4 mg/kg). These authors also observed that there is no correlation between haloperidol- and SCH 23390-induced catalepsy.

Kanes et al. (1996) determined the strain means (average ED_{50} values) for 25 strains of the BXD RI series and correlated these means with the strain distribution patterns of 1300 marker loci, each mapped to a chromosomal region and showing allelic differences between the C57BL/6 and DBA/2 strains. This process identified six provisional QTLs at $P<0.01$. Two of these QTLs (one near the *b* locus on chromosome 4 and one near *Drd2* on chromosome 9) were confirmed at $P<0.01$ or better in 144 B6D2 F_2 individuals phenotyped for haloperidol-induced catalepsy and genotyped using microsatellite markers. At the *b* locus the D_2 allele is associated with enhanced response while at the *Drd2* locus, the D_2 allele is associated with non-response. These relationships were also confirmed in a new sample of 470 F2 individuals by noting significant associations between haloperidol response and brown coat color ($P<0.01$) and dilute coat color ($P<0.03$). The d locus is located distal to the Drd2 on chromosome 9 and near Htlb. Finally, Rasmussen et al. (unpublished data) examined the relationship between coat color and haloperidol response in 550 F2 animals formed by crossing the BALB/c and LP strains (the extremes of the distribution for inbred strains). The appearance of piebald spotting was significantly enhanced in the haloperidol non-responders ($P<0.0005$). This association is of interest, since the s locus is located near the *Htr2a* serotonin receptor gene.

Anxiolytics

Strain distributions have been described for two standard animal models of anxiety-like behaviors, mouse light↔dark exploration and the elevated plus maze. Both represent approach-avoidance conflicts; both have been optimized for mice.

The mouse light↔dark exploration model of anxiety-related behaviors is based on the ethologically-relevant conflict between the tendency of mice to explore a novel environment, and the aversive properties of a brightly lit open field (Crawley and Goodwin 1980; Crawley 1981). A standard rat cage is divided into two compartments, one large, open, and lighted, the other small, dark, and enclosed. An automated photocell array at the opening between the two compartments measures transitions between the light and dark compartments and the time spent in each. As with many experimental protocols, drugs that affect general motor function will affect light↔dark performance, such that parallel experiments for general locomotion in an automated open field serve as necessary controls. Extensive pharmacological characterization of the light↔dark model has shown that anxiolytic drugs increase exploratory behaviors, as measured by the number of transitions and/or time spent in the dark, while other classes of drugs do not (Crawley and Goodwin 1980; Crawley 1981; Crawley et al. 1981, 1986; Mathis et al. 1994, 1995).

Table 7 Anxiety-related behaviors

Mouse strain	Number of light↔dark transitions[a]	
	Baseline	Response to diazepam
C57BL/6J	49±3	91±11*
Swiss Webster/NIH	38±2	67±4*
DBA	21±2	27±7
CF-1	20±4	26±3
Swiss Webster/Harlan	14±6	24±3
A/J	9±1	10±2
BALB/cJ	8±3	7±5

[a] Modified from Crawley and Davis (1982) and Mathis et al. (1994)

* $P<0.01$. Number of light↔dark transitions after diazepam treatment significantly greater than number of light↔dark transitions after vehicle treatment

Strain distribution analysis of four inbred and three outbred strains of mice, on baseline number of light↔dark transitions and on response to diazepam (Valium), is presented in Table 7 (Crawley and Davis 1982; Mathis et al. 1994). Strains of mice which show strong responses to anxiolytics include the C57BL/6J from Jackson Labs and the NIH/s from the National Institutes of Health Veterinary Resources breeding center (Crawley and Davis 1982; Mathis et al. 1994, 1995). Strains with low numbers of baseline transitions generally show weak responses to anxiolytics (Crawley and Davis 1982; Mathis et al. 1994, 1995).

The elevated plus maze is based on a similar conflict between the tendency of mice to explore a novel environment, and the aversive properties of an open, elevated visual cliff (Pellow et al. 1985; Pellow and File 1986; Lister 1987). The elevated plus maze is generally constructed of black Plexiglas, consisting of two open arms and two enclosed arms, extending a right angles from a central platform, with the entire apparatus raised at least 30 cm from the floor. Anxiolytic drugs increase the number of entries into the open arms, as compared to number of entries into the closed arms. Time spent in each arm, and percent time or percent number of entries into each arm, provide additional useful parameters. Percent number of entries into the open arms is considered the best measure of anxiolytic tendencies or lack of fearfulness. Total number of entries into all four arms of the maze provides an independent measure of non-specific drug effects, e.g. a sedating dose of a drug will decrease total number of entries, a psychostimulant will increase total number of entries.

Strain distribution analysis of 16 inbred strains of mice on elevated plus maze behavior was published by Trullas and Skolnick (1993). Rank order of percent entries into the

open arms was BALB/cJ >BALB/cByJ >CBA/J=C3H/HeJ >NIH/Nude >C3H.SW/SnJ >C3H/HeN >DBA/2J >AKR/J >NZB/B1NJ >C57BL/6ByJ >C57BL/6J >B10.BR/SgSnJ >B10.D2/nSnJ >C57BL/10J >A/J. Strain distributions on responses to benzodiazepines in the elevated plus maze have not been conducted to date.

It is interesting to note similarities and differences in the strain distributions between the elevated plus maze and the light↔dark models. The A/J strain was the most anxiogenic-like or fearful, and the DBA strain was moderate on both number of light↔dark transitions and percent entries into the open arms of the elevated plus maze. The C57BL/6J strain was moderate on the elevated plus maze, while exhibiting low fearfulness and a robust anxiolytic response to diazepam on light↔dark transitions. BALB/cJ was the least fearful on the elevated plus maze, while among the most fearful in the light↔dark chamber. Differences in strain distributions between two related animal models of anxiety serve to highlight the fact that each experimental protocol represents a unique set of behaviors, which are regulated by a unique set of environmental, neuroanatomical, neurochemical, neurophysiological, and genetic factors.

For the light↔dark transitions model of anxiety-related behaviors, the C57BL/6J strain is recommended for null mutations predicted to increase, and the A/J strain is recommended for null mutations predicted to decrease, anxiety-like behaviors.

Discussion

Traditionally, genetic approaches toward studying behavior in mice have focused on pre-existing, "natural" genetic differences amongst laboratory inbred strains. These differences are manifest either as single gene mutations with a major influence on trait variance, or as complex traits whereby multiple genes plus environmental factors influence the behavior. Identification of single gene neurological mutations is now possible in mice, and some of these mutations have behavioral manifestations, such as weaver (Patil et al. 1995) and *Atm* (Barlow et al. 1996). However, most natural variants present themselves as complex quantitative traits. Recent advances in molecular and quantitative genetics have made possible the chromosomal mapping of QTLs, the genes that underlie behavioral traits, by relating individual differences to a dense map of genetic markers (Lander and Botstein 1989). Extension of these methods to use existing recombinant inbred strains (Plomin et al. 1991) pioneered a rapid expansion of the QTL method to study behavioral and drug-response traits (e.g., Gora-Maslak et al. 1991; Johnson et al. 1992; Belknap et al. 1993; Flint et al. 1995; Mathis et al. 1995). Yet the molecular identification of the gene(s) involved in complex behavioral traits is still a major challenge, even in today's high-tech atmosphere. Targeted germ line mutations have become a powerful alternative tool for studying the genetic basis of behavior.

The description of behavioral phenotypes of inbred strains of mice, provided above for a variety of behavioral paradigms, is a useful guide for choosing the best inbred strains for studying knockout or transgenic behavioral phenotypes. The principles upon which these summaries are based would readily apply to most other behavioral paradigms. The construction and propogation of single gene mutant mice for behavioral studies should address not only the status of the single gene mutation but also the genetic background. The data described above can be further used to guide breeding strategies and the potential development of new embryonic stem cell lines.

Caveats: environmental effects

Strain differences in most behaviors are environment-dependent. Effects of prenatal, postnatal, preweaning, nutritional, husbandry, and physical environments are known. For example, the presence of an adult male in the postnatal/preweaning period facilitates aggression, and water deprivation of adults decreases aggression (Maxson 1992b). The stress of shipping mice from the supplier to the lab may produce irreversible effects on some behaviors. Strain by environment interactions resulting in long-lasting behavioral changes in mice may explain behavioral differences between mice used directly from a vendor and mice used from the investigator's local breeding colony. It is crucial to use identical environmental and test conditions within the same laboratory environment, to compare behaviors for the mutants, heterozygotes, wild type littermates, and the parental strains used to create and propagate the mutation.

Breeding strategies

Thoughtful breeding strategies can avoid some of the potential confounding problems of unusual background genes. If the strain of the embryonic stem cells is the same as that used in the test cross during the construction of the null mutant, then the strategy is simple. The null mutation is on a fixed inbred background and the colony is maintained as an inbred colony with a single genetic background. The behavioral effect of a knockout or transgenic could be determined on a uniform genetic background. However, when the mutation is transferred to other genetic backgrounds, particularly using a strain with some endogenous behavioral or anatomical abnormality, the issues are more complex. For example, many substrains of 129 mice, including many used for embryonic stem cells in most knockout mice, show an aberrant, incomplete corpus callosum, the main fiber bundle that connects the right and left hemispheres of the cerebral cortex (Livy and Wahlsten 1991). The contribution of an unusual behavioral phenotype from the ES line must be factored into the cross with another strain, such as C57BL/6, at the time of generating chimeras. The F1s

that are heterozygous for the null mutation are bred to produce the F2 population, which will be tested for the phenotype of interest. While the F1 hybrids are isogenic, the F2 mice are not. There will be variability across individuals because independent assortment and recombination produces new combinations of segregating background genes from the two parental lines. These new arrays of background genes may have profound influences on new behavioral phenotypes, or may even synthesize them de novo. To assess the impact of a null mutation, wild-type mice must be compared with mutants within litters, as well as across several litters derived from crosses of F1 mice. The phenotype of these F2 mice might be complicated by unusual background genes. It is virtually impossible to calculate the number of mice that must be tested to represent all possible combinations of important background genes, since this requires prior knowledge of the heritability of a specific phenotype in that particular genetic cross. One robust, though laborious way around this is to examine enough mice to demonstrate a "highly significant" association between marker and phenotype, i.e. standard linkage guidelines (Lander and Schork 1994; Lander and Kruglyak 1995). Alternatively, it may be as helpful to consider how the mutant phenotype compares with that of other common mouse strains. For example, if the phenotype is within the range of normal variation, than a synthetic background effect would be more difficult to exclude. In general, because the animal costs behind doing these experiments properly may be prohibitive to many investigators, such common sense methods are useful.

Establishing a breeding colony for a knockout mutant can be conducted at the F2 stage. If viable offspring can be generated from crossing homozygotes, then separate homozygous lines and wild-type lines might be generated. However, this could produce inbreeding of background genes which influence the polygenic traits to be analyzed. Using a large number of families established at this point will increase the probability of preserving random combinations of background genes, resulting in a better assessment of the impact of the null mutation.

Another short-term strategy is to breed heterozygotes from different founder families to derive homozygotes, heterozygotes, and wild-types for behavioral comparisons. To keep the probability low for unwanted inbreeding of background genes, heterozygotes can be generated from heterozygote wild type crosses. Clearly, the greater the number of breeders the better. The breeding colony should be maintained in subsequent generations by breeding only animals that are distant relatives. For example, never breed animals that have common grandparents.

The best long-term strategy is the creation of congenics. To retain the single gene mutation on a fixed genetic background, the null mutation is usually moved to a standard inbred background through systematic backcrosses, to create a congenic line (for a description of congenics, see Silver 1995). Considerations for the choice of the recipient inbred strain for the movement of a single gene have been extensively discussed (Smithies and Maeda 1995). Briefly, the size of the DNA segment ultimately transferred in the creation of a congenic is larger than the null mutant gene because of the physical constraints of recombination. Therefore, linked sequences that may influence the phenotype will be bred onto the desired genetic background. Naturally occurring DNA polymorphisms in the gene of interest, or in linked sequences, could affect the phenotype. A separate congenic line for the wild-type gene should also be created by retaining animals created from embryonic stem cells with the wild-type allele of the gene of interest.

A note on nomenclature

From the present review, it is clear that there are large behavioral differences among various inbred strains of mice, which may be used to develop transgenics and knockouts. In addition, there are often significant subtype differences within several of the inbred strains. For example, 129/SvEvTac are good learners in the Morris water task and contextual fear conditioning, and show good startle reflexes, whereas 129/SvJ are poor learners and show reduced startle (see previous sections). Therefore, the strains of mice used for the embryonic stem cells and for breeding need to be stated in every publication. Nomenclature recommended by the International Committee on Standardized Genetic Nomenclature for Mice, and published by The Jackson Laboratory (Web Site http://www.informatics.jax.org/nomen/ and *Jax Notes*, Winter 1996), includes the strains of origin, the designation as F1 hybrid if appropriate, the allele of the targeted mutation, and the stock number from the supplier. For example, B6,129-Apoe$^{tm1Unc/129}$ (Jackson Stock #1000) denotes the first targeted mutation of the *Apoe* gene made in 129 ES cells at the University of North Carolina and maintained on an inbred strain whose genetic background is derived from C57BL/6 and 129 strains at Jackson Laboratory, fictitious stock #1000.

Embryonic stem cell lines

Virtually all ES lines for gene targeting are made from substrains of 129 mice. The 129 strains have proven especially useful for creating germline competent ES lines. Since it is an arduous task to develop each knockout, investigators rely heavily on technologies with proven success, and 129-based ES lines have an impressive record of success. Undoubtedly, the preponderance of knockouts will continue to be made with embryonic stem cells from 129 substrains. Behavioral geneticists studying a knockout or transgenic mouse derived from a 129 substrain need to evaluate the performance of the particular substrain of 129 in each relevant behavioral test, especially where substrain performance on that task has not been previously reported. As shown above, there are wide performance variations among 129 substrains for learning and memory, startle, and prepulse inhibition.

Table 8 Comparison of inbred mouse strains C57BL/6 and DBA/2 on behavioral phenotypes

Behavior	Strain	
	C57BL/6	DBA/2
Open field activity[a]	High	Moderate
Learning (Morris water task)[b]	Good	Poor
Male offense aggression[c]	Low	High
Copulation[d]	High	Low
Parental behaviors[e]	Low	Moderate
Acoustic startle[f]	Moderate	Poor
Prepulse inhibition[f]	Low	Moderate
Anxiety[a,g]	Low	Moderate
Ethanol ingestion[h]	High	Low
Cocaine self-administration[i]	High	Low
Morphine locomotor activation[j]	High	Low
Haloperidol-induced catalepsy[k]	Low	High

[a] Crabbe (1986)
[b] Upchurch and Wehner (1989)
[c] Selmanoff et al. (1976)
[d] McGill (1970)
[e] Carlier et al. (1982)
[f] Paylor and Crawley (1996)
[g] Crawley and Davis (1982)
[h] Belknap et al. (1993)
[i] Seale (1991)
[j] Belknap and O'Toole (1991)
[k] Kanes et al. (1993)

Further behavioral analyses of 129 substrains will likely provide investigators with information on 129 substrains that have general utility for behavioral tasks, and may reveal particular strengths and weaknesses of 129 substrains in individual tasks.

Specialized ES lines have been created by investigators that have specific research interests. C57BL/6J (Lederman and Burki 1991; Wiles and Keller 1991) and CD-1 (Suda et al. 1987) lines have been developed. The germline potential of these lines has not be tested extensively, nor have gene targeting rates been evaluated systematically. Therefore, the general utility of these lines for the creation of knockouts remains to be determined. Of note, F1 hybrid ES lines have been produced between 129 and C57BL/6J with germline potential after genetic manipulation (Martin 1981; R. Jaenisch, personal communication).

The behavioral geneticists authoring this article have reached a consensus that the most useful inbred strains for new ES lines would be the C57BL/6J and the DBA/2J. This opinion is based on the enormous literature describing these two strains on a great many behavioral paradigms, described in detail above, and partially summarized in Table 8. It would be useful to make disruptions of important behavioral genes directly in ES lines made from these strains. This will require testing of available cell lines, or the creation of new cell lines, from these strains. If successful ES lines are derived from these two strains, the behavioral and molecular genetics communities will have powerful new tools for creating knockout mouse lines to analyze the the genetic determinants of complex behaviors.

The future: conditional knockouts

Conditional knockouts are designed to restrict the effects of the mutation to a specific period of adulthood, avoiding the complications of genetic compensation during development, and/or to restrict the effects of the mutation to one cell type, avoiding the complications of expression of the mutation throughout the brain and body. Currently, several laboratories are refining techniques that will allow more precise alteration of gene expression than the all-or-none alleles created at the blastocyst stage of development by standard transgenic and knockout technology. Conditional knockouts, using Cre/LoxP mediated recombination (Sauer 1993; Tsien et al. 1996) and tetracycline activation and repression of expression (Gossen and Bujard 1972; Mayford et al. 1996) hold great promise in the future for behavioral genetics. Such exquisite control of gene expression will allow investigators to use the most appropriate controls for behavioral experiments, i.e. the same mouse with and without expression of a gene.

Until the conditional knockout technology is further refined and routinely available, much can be learned from behavioral analysis of transgenic and knockout mice as they are produced today, as long as issues such as genetic background are properly taken into consideration. An extremely exciting aspect of this technology is its therapeutic implications. Transgenic and knockout mouse models of human diseases are now being used to develop effective gene therapy strategies for human genetic disorders. Investment now, in choosing the best progenitor strains for each mutation, will pay off later in optimized animal models for the testing of new treatments for genetic diseases.

Conclusion: choosing the best strain

The specific hypothesis being tested determines the right mouse strain for a new transgenic or knockout experiment. The information provided in the present review summarizes findings of inbred mouse strain distributions for a wide variety of behavioral paradigms and scientific questions. Molecular biologists and behavioral neuroscientists are encouraged to consult the referenced literature, at the beginning stages of their collaboration, to choose the optimal strain whose background genes will be most appropriate for the specific targeted null mutation, and for the specific behavioral paradigm. There is no "best" strain that can be recommended across all behavioral paradigms for all null mutations. Rather, the strain is chosen for its predicted sensitivity to the null mutation. High-scoring strains will be ideal to detect a null mutation postulated to reduce the behavioral phenotype; low-scoring strains will be ideal for null mutations postulated to elevate the behavioral phenotype; moderate-scoring strains will be ideal when the effects of the null mutation could go either way. The present authors hope that this article will serve to guide investigators to the wealth of available knowledge in behavioral genetics, to make an informed choice of the best inbred strain for the development of a new null mutation.

Acknowledgements Preparation of this paper was supported by grants to the individual authors, including USPHS AA10760, AA06243, AG13622-01, DA-00197, DA-03194, DA05228, HD33098-01A1, MH48663, and MH53668, and from the Department of Veterans Affairs, Whitehall Foundation, Beckman Foundation, Klingenstein Foundation, McKnight Foundation, Merck Foundation, Neurofibromatosis Foundation, and Neurofibromatosis Consortium.

This publication was initiated through the Workshop on Behavioral Phenotypes of Inbred Strains of Mice, J.N.Crawley and R. Paylor, Organizers, at The Cloisters, National Institutes of Health campus in Bethesda, Md., USA, on April 24th, 1996. Support for the preparation of this manuscript was provided by the Workshop spunsors, the National Institute of Mental Health Intramural Research Program and the National Human Genome Research Institute Intramural Research Program. Co-sponsors included the National Institute on Alcohol Abuse and Alcoholism Intramural Research Program, National Institute on Alcohol Abuse and Alcoholism Program in Genetics, National Institute on Child Health and Human Development Intramural Research Program, National Institute on Drug Abuse, National Institute of Neurological Disorders and Stroke Intramural Research Program, and the National Institutes of Health Office of Behavioral and Social Sciences Research. We thank Dr. David Pickar, Chief, Experimental Therapeutics Branch, National Institute of Mental Health, for his support and encouragement in the conceptual development of this manuscript.

References

Ammassari-Teule M, Caprioli (1985) Spatial learning and memory, maze running strategies and cholinergic mechanisms in two inbred strains of mice. Behav Brain Res 17:9–16

Ammassari-Teule M, Hoffman H-J, Rossi-Arnaud C (1993) Learning in inbred mice: strain-specific abilities across three radial maze problems. Behav Genet 23:405–412

Archer J (1973) Tests for emotionality in rats and mice: a review. Anim Behav 21:205–235

Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, Shiloh Y, Crawley JN, Ried T, Tagle D, Wynshaw-Boris A (1996) Atm-deficient mice: a paradigm of ataxia-telangiectasia. Cell 86:159–171

Batty J (1978) Plasma levels of testosterone and male sexual behaviour in strains of the house mouse (*Mus musculus*). Anim Behav 26:339–348

Belknap JK (1980) Genetic factors in the effects of alcohol: neurosensitivity, functional tolerance and physical dependence. In: Rigter H, Crabbe JC (eds) Alcohol tolerance and dependence. Elsevier, Amsterdam, pp 157–180

Belknap JK, O'Toole LA (1991) In: Crabbe JC, Harris RA (eds) The genetic basis of alcohol and drug actions. Plenum Press, New York, pp 225–252

Belknap JK, Crabbe JC, Young ER (1993a) Voluntary consumption of ethanol in 15 inbred mouse strains. Psychopharmacology 112:352–358

Belknap JK, Metten P, Helms ML, O'Toole LA, Angeli-Gade S, Crabbe JC, Phillips TJ (1993b) Quantitative trait loci (QTL) applications to substances of abuse: physical dependence studies with nitrous oxide and ethanol in BXD mice. Behav Genet 23:213–222

Belknap JK, Mogil JS, Helms ML, Richards SP, O'Toole LA, Bergeson SE, Buck KJ (1995) Localization to chromosome 10 of a locus influencing morphine-induced analgesia in crosses derived from C57BL/6 and DBA/2 mice. Life Sci 57:PL117–PL124

Berrettini WH, Ferraro TN, Alexander RC, Buchberg AM, Vogel WH (1994) Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains. Nature Genet 7:54–58

Bindra D, Thompson WR (1953) An evaluation of defecation and urination as measures of fearfulness. J Comp Physiol Psychol 46:43–45

Braff DL, Stone C, Callaway E, Geyer MA, Glick ID, Bali L (1978) Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology 15:339–343

Brain PF (1978) Hormones, drugs and aggression. vol. 3. Eden, Exeter

Broadhurst PL (1978) Drugs and the inheritance of behavior. Plenum Press, New York

Brodda J, Svare B (1982) Postpartum aggression in C57BL/6J and DBA/2J mice: experiential and environmental influences. Behav Neural Biol 35:76–83

Bullock AE, Slobe BS, Collins AC (1995) Classical genetic analysis of startle and prepulse inhibition of startle in mice. Abstr Soc Neurosci 21:199

Buselmaier W, Vierling T, Balzereit W, Schwelger H (1981) Genetic analysis of avoidance learning by means of different psychological testing systems with inbred mice as model organisms. Psychol Res 43:317–333

Carlier M, Roubetoux P, Cohen-Salmon Ch (1982) Differences in patterns of pup care in *Mus musculus domesticus* I – comparisons between eleven inbred strains. Behav Neural Biol 35:205–210

Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Poumin S, Muller U, Aguet M, Babinet C, Shih JC, De Maeyer E (1995) Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science 268:1763–1766

Cohen-Salmon Ch (1988) What role does sensory perception play in the onset and maintenance of pup care behavior in laboratory rodents? Eur Bull Cognit Psychol 8:53–94

Cohen-Salmon Ch, Carlier M, Roubertoux P (1982) Differences in patterns of pup care in *Mus musculus domesticus* II – effects of parity on eight inbred strains. Biol Behav 7:337–346

Cohen-Salmon Ch, Carlier M, Roubertoux J, Jouhaneau C, Semal C, Paillette M (1985) Differences in patterns of pup care V-pup ultrasonic emissions and pup care behavior. Physiol Behav 35:167–174

Crabbe JC (1986) Genetic differences in locomotor activity in mice. Pharmacol Biochem Behav 25:289–292

Crabbe JC, Janowsky J, Young E, Kosobud A, Stack J, Rigter H (1982) Tolerance to ethanol hypothermia in inbred mice: genotypic correlations with behavioral responses. Alcohol Clin Exp Res 6:446–458

Crabbe JC, Young ER, Kosobud A (1983) Genetic correlations with ethanol withdrawal severity. Pharmacol Biochem Behav 18 [Suppl 1]:541–547

Crabbe JC, Belknap JK, Mitchell SR, Crawshaw LI (1994a) Quantitative trait loci mapping of genes that influence the sensitivity and tolerance to ethanol-induced hypothermia in BXD recombinant inbred mice. J Pharmacol Exp Ther 269:184–192

Crabbe JC, Gallaher ES, Phillips TJ, Belknap JK (1994b) Genetic determinants of sensitivity to ethanol in inbred mice. Behav Neurosci 108:186–195

Crabbe JC, Phillips TJ, Gallaher EJ, Crawshaw LI, Mitchell SR (1996) Common genetic determinants of ataxic and hypothermic effects of ethanol in BXD/Ty recombinant inbred mice: genetic correlations and quantitative trait loci. J Pharmacol Exp Ther 277:624–632

Crawley JN (1981) Neuropharmacological specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol Biochem Behav 15:695–699

Crawley JN (1996) Unusual behavioral phenotypes in inbred mouse strains. Trends Neurosci 19:181–182

Crawley JN, Davis LG (1982) Baseline exploratory activity predicts anxiolytic responsiveness to diazepam in five mouse strains. Brain Res Bull 8:609–612

Crawley JN, Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13:167–170

Crawley JN, Marangos PJ, Paul SM, Skolnick P, Goodwin FK (1981) Purine diazepam interaction: inosine reverses diazepam-induced stimulation of mouse exploratory behavior. Science 22:725–727

Crawley JN, Glowa JR, Majewska MD, Paul SM (1986) Anxiolytic activity of an endogenous adrenal steroid. Brain Res 398:382–385

Dains K, Hitzemann B, Hitzemann R (1996) Genetics, neuroleptic-response and the organization of cholinergic neurons in the mouse striatum. J Pharmacol Exp Ther 279:1430–1438
deFiebre CM, Ruth JA, Collins AC (1989) Differential sensitivity of long-sleep and short-sleep mice to high doses of cocaine. Pharmacol Biochem Behav 34:887–893
DeFries JC, Gervais MC, Thomas EA (1978) Response to 30 generations of selection for open-field activity in laboratory mice. Behav Genet 8:3–13
Deitrich RA, Spuhler K (1984) Genetics of alcoholism and alcohol actions. In: Smart RG, et al. (eds) Research advances in alcohol and drug problems, 8. Plenum Press, New York, pp 47–98
Fanselow MS (1990) Factors governing one trial context conditioning. Anim Learn Behav 18:264–270
Flint J, Corley R, DeFries JC, Fulker DW, Gray JA, Miller S, Collins AC (1995) A simple genetic basis for a complex psychological trait in laboratory mice. Science 269:1432–1435
Franks RD, Adler LE, Waldo MC, Alpert J, Freedman R (1983) Neurophysiological studies of sensory gating in mania: comparison with schizophrenia. Biol Psychiatry 18:989–1005
Freedman R, Adler LE, Waldo MC, Pachtman E, Franks RD (1983) Neurophysiological evidence for a defect in inhibitory pathways in schizophrenia: comparison of medicated and drug-free patients. Biol Psychiatry 18:537–552
Freedman R et al. (1987) Neurobiological studies of sensory gating in schizophrenia. Schizophr Bull 13:669–677
Fuller JL (1964) Measurement of alcohol preference in genetic experiments. J Comp Physiol Psychol 57:85–88
Gallaher EJ, Jones GE, Belknap JK, Crabbe JC (1966) Identification of genetic markers for initial sensitivity and rapid tolerance to ethanol-induced ataxia using quantitative trait locus analysis in BXD recombinant inbred mice. J Pharmacol Exp Ther 277:604–612
George FR (1989) Cocaine produces low dose locomotor depressant effects in mice. Psychopharmacology 99:147–150
George FR, Goldberg SR (1989) Genetic approaches to the analysis of addiction processes. Trends Pharmacol Sci 10:78–83
George FR, Ritz MC (1991) Cocaine produces locomotor stimulation in SS/Ibg but not LS/Ibg mice. Psychopharmacology 101:18–22
Gerlai R (1996) Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? Trends Neurosci 19:177–181
Geyer MA, Swerdlow NR, Mansbach RS, Braff DL (1990) Startle response models of sensorimotor gating and habituation deficits in schizophrenia. Brain Res Bull 25:485–498
Ginsburg BE, Allee WC (1942) Some effects of conditioning on social dominance and subordination in inbred strains of mice. Physiol Zool 15:495–506
Giros B, Jaber M, Jones SR, Wightman RM, Caron MG (1996) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379:606–612
Gora-Maslak G, McClearn GE, Crabbe JC, Phillips TJ, Belknap JK, Plomin R (1991) Use of recombinant inbred strains of identify quantitative trait loci in psychopharmacology. Psychopharmacology 104:413–424
Gossen M, Bujard H (1992) Tight control of gene expression in mammalian cells by tetracycline responsive promoters. Proc Natl Acad Sci USA 89:5547–5551
Graham FK (1975) The more or less startling effects of weak prestimulation. Psychophysiology 12:238–248
Grahame NJ, Phillips TJ, Burkhart-Kasch S, Cunningham CL (1995) Intravenous cocaine self-administration in the C57BL/6J mouse. Pharmacol Biochem Behav 51:827–834
Guillot P-V, Roubertoux PL, Crusio WE (1994) Hippocampal mossy fiber distributions and intermale aggression in seven inbred mouse strains. Brain Res 660:167–169
Hall CS (1934) Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. J Comp Psychol 18:385–403
Hall CS (1936) Emotional behavior in the rat. III. The relationship between emotionality and ambulatory activity. J Comp Psychol 22:345–352
Haug M, Johnson FJ, Brain PF (1992) Biological correlates of attack on lactating intruders by female mice: a topical review. In: Bjorkqvist K, Niemela P (eds) Of mice and women: aspects of female aggression. Academic Press, New York, pp 381–393
Hay D (1985) Essentials of behavior genetics. Blackwell, Melbourne
Henderson ND (1967) Prior treatment effects on open field behavior of mice: a genetic analysis. Anim Behav 15:364–376
Henderson ND (1986) Predicting relationships between psychological constructs and genetic characters: an analysis of changing genetic influences on activity in mice. Behav Genet 16:201–220
Henderson ND (1989) Genetic analysis of an avoidance-avoidance response in *Mus domesticus*. Behav Genet 19:387–407
Hewitt JK, Broadhurst PL (1983) Genetic architecture and the evolution of aggressive behavior. In: Simmel EC, Hahn ME, Walters JK (eds) Aggressive behavior: genetic and neural approaches. Erlbaum, New York, pp 37–66
Hitzemann RJ, Dains K, Bier-Langing CM, Zahniser NR (1991) On the selection of mice for haloperidol response and non-response. Psychopharmacology 103:244–250
Horowitz GP, Dudek BC (1983) Behavioral pharmacogenetics. In: Fuller JL, Simmel EC (eds) Behavior genetics: principles and applications. Erlbaum, Hillsdale, N.J., pp 117–154
Johansson C, Jackson DM, Zhang J, Svensson L (1995) Prepulse inhibition of acoustic startle, a measure of sensorimotor gating: effects of antipsychotics and other agents in rats. Pharmacol Biochem Behav 52:649–654
Johnson TE, DeFries JC, Markel PD (1992) Mapping quantitative trait loci for behavioral traits in the mouse. Behav Genet 22:635–653
Jones BC, Campbell AD, Radcliffe RA, Erwin VG (1991) Cocaine actions, brain levels and receptors in selected lines of mice. Pharmacol Biochem Behav 40:941–948
Jones SE, Brain PF (1987) Performance of inbred and outbred laboratory mice in putative tests of aggression. Behav Genet 17:87–96
Kanes SJ, Hitzemann BA, Hitzemann RJ (1993) On the relationship between D_2 receptor density and neuroleptic-induced catalepsy among eight inbred strains of mice. J Pharmacol Exp Ther 267:538–547
Kanes SJ, Dains K, Cipp L, Gatley J, Hitzemann B, Rasmussen E, Sanderson S, Silverman M, Hitzemann R (1996) Mapping the genes for haloperidol-induced catalepsy. J Pharmacol Exp Ther 277:1016–1025
Kulikov A, Popova N (1996) Association between intermale aggression and genetically-defined tryptophan hydroxylase activity in the mouse brain. Aggress Behav 22:111–117
Lagerspetz KMJ, Lagerspetz KYH (1974) Genetic determination of aggressive behaviour. In: Van Abeelen JHF (ed) The genetics of behavior. Elsevier, New York, pp 321–346
Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–189
Lander ES, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genet 11:241–247
Lander ES, Schork NJ (1994) Genetic dissection of complex traits. Science 265:2037–2048
Lederman B, Burki K (1991) Establishment of a germline competent C57BL/6 embryonic stem cell line. Exp Cell Res 197:254–258
Lipp H-P, Schwegler H, Crusio WE, Wolfer DP, Leisinger-Trigona M-C, Heimrich B, Driscoll P (1989) Using genetically-defined rodent strains for the identification of hippocampal traits relevant for two-way avoidance behavior: a non-invasive approach. Experientia 45:845–859
Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92:180–185
Livy DJ, Wahlsten D (1991) Tests of genetic allelism between four inbred mouse strains with absent corpus callosum. J Hered 82:459–464

Marks MJ, Stitzel JA, Collins AC (1989a) Genetic influences on nicotine responses. Pharmacol Biochem Behav 33:667–678
Marks MJ, Romm E, Campbell SM, Collins AC (1989b) Variation of nicotinic binding sites among inbred strains. Pharmacol Biochem Behav 33:679–689
Marks MJ, Campbell SM, Romm E, Collins AC (1991) Genotype influences the development of tolerance to nicotine in the mouse. J Pharmacol Exp Ther 259:392–402
Marley RJ, Witkin JM, Goldberg SR (1991) Genetic factors influence changes in sensitivity to the convulsant properties of cocaine following chronic treatment. Brain Res 542:1–7
Martin GR (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA 78:7634–7638
Mathis C, Paul SM, Crawley JN (1994) Characterization of benzodiazepine-sensitive behaviors in the A/J and the C57BL/6J inbred strains of mice. Behav Genet 24:171–180
Mathis C, Neumann PE, Gershenfeld H, Paul SM, Crawley JN (1995) Genetic analysis of anxiety-related behaviors and responses to benzodiazepine-related drugs in AXB and BXA recombinant inbred mouse strains. Behav Genet 25:557–568
Maxson SC (1981) The genetics of aggression in vertebrates. In: Brain PF, Benton D (eds) The biology of aggression. Sijthoff and Noordhoff, The Netherlands, pp 69–104
Maxson SC (1992a) Potential genetic models of aggression and violence in males. In: Driscoll P (ed) Genetically defined animal models of neurobehavioral dysfunctions. Birkhauser, Boston, pp 174–188
Maxson SC (1992b) Methodological issues in genetic analyses of an agonistic behavior (offense) in male mice. In: Goldowitz D, Wahlstein D, Wimer RE (eds) Techniques for the genetic analyses of brain and behavior: focus on the mouse. Elsevier, New York, pp 349–373
Maxson SC, Shrenker P, Vigue LC (1983) Genetics, hormones and aggression. In: Svare B (ed) Hormones and aggressive behaviour. Plenum Press, New York, pp 179–196
Mayford M, Bach ME, Huang Y-Y, Wang L, Hawkins RD, Kandel ER (1996) Controls of memory formation through regulated expression of a CaMKII transgene. Science 274:1678–1683
McClearn GE, Rodgers DA (1959) Differences in alcohol preference among inbred strains of mice. Q J Stud Alcohol 20:691–695
McGill TE (1962) Sexual behavior in three inbred strains of mice. Behaviour 19:341–350
McGill TE (1970) Genetic analysis of male sexual behavior. In: Lindzey G, Thiessen DD (eds) Contributions to behavior-genetic analysis: the mouse as a prototype. Appleton-Century-Crofts, New York, pp 57–88
McGill TE, Ransom TW (1968) Genotypic change affecting conclusions regarding the mode of inheritance of elements of behavior. Anim Behav 16:88–91
Metten P, Crabbe JC (1994) Common genetic determinants of severity of withdrawal from ethanol, pentobarbital and diazepam in inbred mice. Behav Pharmacol 5:533–547
Michard C, Cartier M (1985) Les conduites d'agression intraspecifique cez la souris domestique: differences individuelles et analyses genetique. Biol Behav 10:123–146
Miner LL (1997) Cocaine reward and locomotor activity in C57BL/6J and 129/SvJ inbred mice and their F1 cross. Pharmacol Biochem Behav (in press)
Miner LL, Collins AC (1989) Strain comparison of nicotine-induced seizure sensitivity and nicotinic receptors. Pharmacol Biochem Behav 33:469–475
Miner LL, Marley RJ (1995a) Chromosomal mapping of the psychomotor stimulant effects of cocaine in BXD recombinant inbred mice. Psychopharmacology 122:209–214
Miner LL, Marley RJ (1995b) Chromosomal mapping of loci influencing sensitivity to cocaine-induced seizures in BXD recombinant inbred strains of mice. Psychopharmacology 117:62–66
Mogil J, Sternberg W, Marek P, Sadowski B, Belknap J, Liebeskind J (1996) The genetics of pain and pain inhibition. Proc Natl Acad Sci USA 93:3048–3055
Morris RGM (1981) Spatial localisation does not depend on the presence of local cues. Learn Motiv 12:239–260
Mosig DW, Dewsbury DA (1976) Studies of the copulatory behavior of house mice (*Mus musculus*). Behav Biol 16:463–473
Nelson RJ, Demas GE, Huang PL, Fishman MC, Dawson VL, Dawson TM, Snyder SH (1995) Behavioral abnormalities in male mice lacking neuronal nitric oxide synthase. Nature 378:383–386
Ogawa S, Makino J (1981) Maternal aggression in inbred strains of mice: effects of reproductive states. Jpn J Psychol 52:78–84
Ogawa S, Makino J (1984) Aggressive behavior in inbred strains of mice during pregnancy. Behav Neural Biol 40:195–204
Ogawa S, Robbins A, Kumar N, Pfaff DW, Sundaram K, Bardin CW (1996) Effects of testosterone and 7α-O-methyl-19-nortestosterone (MENT) on sexual and aggressive behaviors in two inbred strains of male mice. Horm Behav 30:74–78
Ogawa S, Lubahn DB, Korach KS, Pfaff DW (1997) Behavioral effects of estrogen receptor gene disruption in male mice. Proc Natl Acad Sci USA 94:1476–1481
Owen EH, Logue SF, Rasmussen DL, Wehner JM (1997) Assessment of learning by the Morris water task and fear conditioning in inbred mouse strains and F1 hybrids: implications of genetic background for single gene mutations and quantitative trait loci analyses. Neuroscience (in press)
Patil N, Cox DR, Bhat D, Faham M, Myers RM, Peterson AS (1995) A potassium channel mutation in weaver mice implicates membrane excitability in granule cell differentiation. Nature Genet 11:126–129
Paylor R, Crawley JN (1997) Inbred strain differences in prepulse inhibition of the mouse startle response. Psychopharmacology (in press)
Paylor R, Baskall L, Wehner JM (1993) Behavioral dissociations between C57BL/6 and DBA/2 mice on learning and memory tasks: a hippocampal-dysfunction hypothesis. Psychobiology 21:11–26
Paylor R, Tracy R, Wehner JM, Rudy JW (1994) DBA/2 and C57BL/6 mice differ in contextual fear but not auditory fear conditioning. Behav Neurosci 108:810–817
Pellow S, File SE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 24:525–529
Pellow S, Chopin P, File SE, Briley M (1985) Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167
Phillips RG, LeDoux JE (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274–285
Phillips TJ, Crabbe JC (1991) Behavioral studies of genetic differences in alcohol action. In: Crabbe JC, Harris RA (eds) The genetic basis of alcohol and drug action. Plenum Press, New York, pp 25–104
Phillips TJ, Lessov CN, Harland RD, Mitchell SR (1996) Evaluation of potential genetic associations between ethanol tolerance and sensitization in BXD/Ty recombinant inbred mice. J Pharmacol Exp Ther 277:613–623
Plomin R, McClearn GE, Gora-Maslak G, Neiderhiser JM (1991) Use of recombinant inbred strains to detect quantitative trait loci associated with behaviour. Behav Genet 21:99–116
Reinstein DK, DeBoissier T, Robinson N, Wurtman RJ (1983) Radial maze performance in three strains of mice: role of the fimbria/fornix. Brain Res 263:172–176
Robinson SF, Marks MJ, Collins AC (1996) Inbred mouse strains vary in oral self-selection of nicotine. Psychopharmacology 124:332–339
Roullet P, Lassalle JM (1990) Genetic variation, hippocampal mossy fibres distribution, novelty reactions and spatial representation in mice. Behav Brain Res 41:61–69
Roullet P, Lassale JM (1995) Radial maze learning using exclusively distant visual cues reveals learners and nonlearners among inbred mouse strains. Physiol Behav 58:1189–1195
Ruth JA, Ullman EA, Collins AC (1988) An analysis of cocaine effects on locomotor activities and heart rate in four inbred mouse strains. Pharmacol Biochem Behav 29:157–162

Saudou FD, Amara A, Dierich A, LeMeur M, Ramboz S, Segu L, Buhot M-C, Hen R (1994) Enhanced aggressive behavior in mice lacking 5HT1B receptor. Science 265:1875–1878
Sauer B (1993) Manipulation of transgenes by site-specific recombination: use of Cre-recombinase. Methods Enzymol 225:890–900
Schneider CW, Evans SK, Chenoweth MB, Beman FL (1973) Ethanol preference and behavioral tolerance in mice: biochemical and neurophysiological mechanisms. J Comp Physiol Psychol 82:466–474
Scott JP (1942) Genetic differences in the social behavior of inbred strains in mice. J Hered 33:11–15
Scott JP (1966) Agonistic behavior of mice and rats: a review. Am Zool 6:683–701
Seale TW (1991) Genetic differences in response to cocaine and stimulant drugs. In: Crabbe JC, Harris RA (eds) The genetic basis of alcohol and drug actions. Plenum Press, New York, pp 279–321
Seale TW, Carney JM (1991) Genetic determinants of susceptibility to the rewarding and other behavioral actions of cocaine. J Addict Dis 10:141–162
Selmanoff MK, Maxson SC, Ginsburg BE (1976) Chromosomal determinants of intermale aggressive behavior in inbred mice. Behav Genet 6:53–69
Selmanoff MK, Abreu E, Goldman BD, Ginsburg BE (1977) Manipulation of aggressive behaviors in adult DBA/2Bg and C57BL/10Bg male mice implanted with testosterone in silastic tubing. Horm Behav 8:377–390
Shuster L, Yu G, Bates A (1977) Sensitization to cocaine stimulation in mice. Psychopharmacology 52:185–190
Shuster L, Garhart CA, Powers J, Grumfeld Y, Kanel G (1988) Hepatotoxicity of cocaine. NIDA Res Monogr 88:250–275
Silver LM (1995) Mouse genetics: concepts and applications. Oxford University Press, New York
Simon NG (1979) The genetics of intermale aggression in mice: recent research and alternative strategies. Neurosci Biobehav Rev 3:97–106
Smithies O, Maeda N (1995) Gene targeting approaches to complex genetic diseases: atherosclerosis and essential hypertension. Proc Natl Acad Sci USA 92:5266–5272
Suda Y, Suzuki M, Ikawa Y, Aizawa S (1987) Mouse embryonic stem cells exhibit indefinite proliferative potential. J Cell Physiol 133:197–201
Svare B (1988) Genotype modulates the aggression-promoting quality of progesterone in pregnant mice. Horm Behav 22:90–99
Swerdlow NR, Geyer MA (1993) Clozapine and haloperidol in an animal model of sensorimotor gating deficits in schizophrenia. Pharmacol Biochem Behav 44:741–744
Swerdlow NR, Keith VA, Braff DL, Geyer MA (1991) Effects of spiperone, raclopride, SCH 23390 and clozapine on apomorphine inhibition of sensorimotor gataing of the startle response in the rat. J Pharmacol Exp Ther 256:530–536
Swerdlow RN, Braff DL, Taaid N, Geyer MA (1994a) Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. Arch Gen Psychiatry 51:139–154
Swerdlow NR, Zisnok D, Taaid N (1994b) Seroquel (ICI 204,636) restores prepulse inhibition of acoustic startle in apomorphine-treated rats: similarities to clozapine. Psychopharmacology 114:675–678
Taylor BA (1972) Genetic relationships between inbred strains of mice. J Hered 63:83–86
Thompson ML, Shuster L, Casey E, Kanel GC (1984) Sex and strain differences in response to cocaine. Biochem Pharmacol 33:1299–1307
Tolliver BK, Carney JM (1994) Sensitization to stereotypy in DBA/2J but not C57BL/6J mice with repeated cocaine. Pharmacol Biochem Behav 48:169–173
Tolliver BK, Belknap JK, Woods WE, Carney JM (1994) Genetic analysis of sensitization and tolerance to cocaine. J Pharmacol Exp Ther 270:1230–1238
Trullas R, Skolnick P (1993) Differences in fear motivated behaviors among inbred mouse strains. Psychopharmacology 111:323–331
Tsien JZ, Chen DF, Gerber D, Tom C, Mercer EH, Anderson DJ, Mayford M, Kandel ER, Tonegawa S (1996) Subregion- and cell type-restricted gene knockout in mouse brain. Cell 87:1317–1326
Upchurch M, Wehner JM (1989) Inheritance of spatial learning ability in inbred mice: a classical genetic analysis. Behav Neurosci 103:1251–1258
Vale JR, Ray D (1972) A diallel analysis of male sex behavior. Behav Genet 2:199–209
Van Abeelen JH (1977) Rearing responses and locomotor activity in mice: single locus control. Behav Neural Biol 19:401–404
Wahlsten D (1972) Genetic experiments with animal learning: a critical review. Behav Biol 7:143–182
Walsh RN, Cummins RA (1976) The open field test: a critical review. Psychol Bull 83:482–504
Weinberger SB, Koob GF, Martinez JL Jr (1992) Differences in one-way active avoidance learning by means of different psychological testing systems with inbred mice as model organisms. Behav Genet 22:177–188
Wiener HL, Reith MEA (1990) Correlation between cocaine-induced locomotion and cocaine disposition in the brain among four inbred strains of mice. Pharmacol Biochem Behav 36:699–701
Wiles M, Keller G (1991) Multiple hematopoetic lineages develop from embryonic stem (ES) cell lines in culture. Development 111:259–267
Willot J, Carlson S, Chen H (1994) Prepulse inhibition of the startle response in mice: relationship to hearing loss and auditory system plasticity. Behav Neurosci 108:703–713
Womer DE, Jones BC, Erwin VG (1994) Characterization of dopamine transporter and locomotor effects of cocaine, GBR 12909, epidepride and SCH 23390 in C57BL and DBA mice. Pharmacol Biochem Behav 48:327–3335
Workshop on Behavioral Phenotypes of Inbred Strains of Mice, at The Cloisters, National Institutes of Health campus, Bethesda, MD, April 24th, 1996